Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

A 31.3
R 31An
Pop.3
ARS 20-9

ARS 20-9
September 1960

U.S. DEPT. OF AGRICULTURE
LIBRARY
JUN 20 1961
CURRENT SERIAL RECORDS

EXTRA COPY

The Nature and Fate of Chemicals Applied to Soils, Plants, and Animals

A Symposium

a symposium held April 27, 28, and 29, 1960, at the Plant Industry Station and Agricultural Research Center Beltsville, Maryland

sponsored by

Farm Research
Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

PREFACE

This publication contains the papers presented at a symposium on agricultural chemicals, sponsored by the Agricultural Research Service, U. S. Department of Agriculture, and held at Beltsville, Maryland, April 27-29, 1960. Some 500 scientists attended, representing Federal research and regulatory agencies, State agricultural experiment stations, and chemical companies.

Symposium speakers discussed new developments in research dealing with agricultural chemicals; the responsibilities of Federal agencies and industry in assuring the safety of chemicals used in producing, processing, and distributing foods; and the potentials for solving current problems in this field through the joint efforts of scientists, regulatory workers, and the chemical and food industries.

It is hoped that both the symposium and the publication of the presentation papers will contribute to greater advancement in research on agricultural chemicals and to a more complete understanding of the part they play in providing our Nation with a wholesome, safe, and abundant food supply.

CONTENTS

Page

SECTION I--RESPONSIBILITY OF THE FEDERAL AGENCIES AND INDUSTRY:

Research in USDA on Chemicals in Agriculture, by T. C. BYERLY 1

Regulatory and Control Programs on Use of Chemicals, by W. L. POPHAM..... 4

Pesticide Residues in Relation to the Food, Drug, and Cosmetic Act, by R. S. ROE 7

Role of Industry in Development and Use of Agricultural Chemicals, by GEORGE R. FERGUSON 10

Agricultural Chemicals in Relation to Human Health, by WAYLAND J. HAYES, JR. 14

SECTION II--PROGRESS AND PROBLEMS:

Use of Herbicides, Growth Regulators, Nematocides, and Fungicides, by W. B. ENNIS, JR 17

Use of Insecticides, Attractants, and Repellents, by E. F. KNIPLING..... 28

Use of Antibiotics, Hormones, Tranquilizers, and Other Chemicals in Animal Production, by N. R. ELLIS, R. E. DAVIS, and C. A. DENTON..... 37

Use of Chemicals for Control of Parasites of Food Animals by A. O. FOSTER, F. D. ENZIE, and D. K. McLOUGHLIN 48

Chemistry of Pesticides in Soils, by L. A. DEAN 63

The Development of Equipment in the Application of Agricultural Chemicals, by WALTER M. CARLETON, L. A. LILJEDAHL, FRANK IRONS, O. K. HEDDEN, and R. D. BRAZEE 70

Use of Insecticides to Protect Stored Grains, Fruits, and Vegetables, by L. S. HENDERSON..... 84

Industrial Chemical Contamination of Livestock Feeds, by AUBREY M. LEE... 92

SECTION III--FATE OF CHEMICALS:

Insecticide Residues in Plants, Animals, and Soils, by W. E. WESTLAKE and J. P. SAN ANTONIO..... 105

Nematocides in Plants and Soils, by A. L. TAYLOR..... 116

Herbicides in Plants, by W. C. SHAW, J. L. HILTON, D. E. MORELAND, and L. L. JANSEN..... 119

The Toxicity of Pesticides for Livestock, by R. D. RADELEFF and R. C. BUSHLAND..... 134

Page

Systemic Insecticides in Animals, by FREDERICK W. PLAPP, JR., WILLIAM F. CHAMBERLAIN, and R. D. RADELEFF 160

Herbicides in Soils, by T. J. SHEETS and L. L. DANIELSON 170

Growth Regulators and Therapeutants...Their Absorption, Translocation, and Metabolism in Plants, by JOHN W. MITCHELL, PAUL J. LINDER, and BERNARD C. SMALE 181

Residues in Milk from Antibiotics Used in Mastitis Treatment, by RICHARD W. BROWN 189

Antibiotics, Hormones, and Tranquilizers in Animals, by J. F. SYKES, J. W. THOMAS, J. T. BITMAN, and C. F. WINCHESTER 204

SECTION I--RESPONSIBILITY OF THE FEDERAL AGENCIES AND INDUSTRY

RESEARCH IN USDA ON CHEMICALS IN AGRICULTURE

T. C. Byerly[1]

The general responsibility of the Department is that for the collection and dissemination of information about all matters pertaining to agriculture, as set forth in the Organic Act when the Department was created in 1862 (5 U.S.C. 511). This authority is:

> "There shall be at the seat of government a Department of Agriculture, the general design and duties of which shall be to acquire and to diffuse among the people of the United States useful information on subjects connected with agriculture in the most general and comprehensive sense of that word, and to procure, propogate, and distribute among the people new and valuable seeds and plants."

The Department achieved Cabinet status on February 9, 1889 (5 U.S.C. 512). More specific research fields were authorized over the years with the creation of bureaus which now comprise the Agricultural Research Service. The Research and Marketing Act of 1946 (P. L. 733, 79th Congress) provided further research into basic laws and principles relating to agriculture and to improve and facilitate the marketing and distribution of agricultural products. This Act specifically authorized contracts and cooperative agreements for research and investigations for utilization and marketing research (7 U.S.C. 427, 1621-1629). Section 10(a), providing for contracts and cooperative agreements for utilization research, is as follows:

> "The Secretary of Agriculture, in accordance with such regulations as he deems necessary, and when in his judgment the work to be performed will be carried out more effectively, more rapidly, or at less cost than if performed by the Department of Agriculture, may enter into contracts with such public or private organizations or individuals as he may find qualified to carry on work under this section without regard to the provisions of section 3709, Revised Statutes, and with respect to such contracts he may make advance progress or other payments without regard to the provisions of section 3648, Revised Statutes. Contracts hereunder may be made for work to continue not more than four years from the date of any such contracts. Notwithstanding the provisions of section 5 of the Act of June 20, 1874, as amended (31 U.S.C. 713), any unexpended balances of appropriations properly obligated by contracting with an organization as provided in this subsection may remain upon the books of the Treasury fund and covered into the Treasury. Research authorized under this subsection shall be conducted so far as practicable at laboratories of the Department of Agriculture. Projects conducted under contract with public and private agencies shall be supplemental to and coordinated with research of these laboratories. Any contracts made pursuant to this authority shall contain requirements making the results of research and investigations available to the public through dedication, assignment to the Government, or such other means as the Secretary shall determine."

[1]Deputy Administrator, Agricultural Research Service, U. S. Department of Agriculture.

Similar authority is provided under section 205(a) for marketing research. This Act was further amended July 28, 1954 (P. L. 545, 83rd Congress) to extend contracting authority for all other fields of agricultural research. The language is as follows:

> "Appropriations for research work in the Department of Agriculture shall be available for accomplishing such purposes by contract through the means provided in subsection (a) hereof."

The annual appropriation act for the Agricultural Research Service under the appropriation language provided the following:

> "Research: For research and demonstrations on the production and utilization of agricultural products, home economics, and related research and services, including administration of payments to State agricultural experiment stations; ($67,722,490 provided by the 1960 appropriation act)."

Of this amount an estimated $6,554,550 will be spent for research related to the safe use of chemicals in agriculture and for the development of biological and other nonchemical methods of pest control.

With respect to agricultural chemicals, the Department must collect and disseminate information that will serve to guide farmers, processors, and distributors, provide advice for its regulatory programs and assurance to the public. Only thus can we continue to enjoy the world's safest, most wholesome, abundant, and excellent food supply.

This general responsibility can be discharged adequately only if the Department maintains a strong intramural research program. While we should and do collect and disseminate research information from all available sources outside the Department, the validity and applicability of such information can be effectively judged by the Department only so long as it has the scientific judgment of its own staff familiar with the area through their own direct research in it.

Recognition of this basic principle is especially important in the field of agricultural chemicals. Since a very large proportion of the research required to develop and test such chemicals is and ought to be conducted by industry, especially on chemicals for which a profitable market is probable, the Department must not neglect the responsibility for verifying a sufficient portion of the procedures and results submitted in substantiation of claims of efficacy, safety, and compliance with legal requirements.

Much of this research and testing is done in cooperation with industry prior to marketing of chemical products for agricultural use. To guide this cooperative research a policy statement has been developed by the Agricultural Research Service. Copies of this statement--Administrative Memorandum 120.4 dated September 18, 1958--may be obtained from the Agricultural Research Service.

But research competence in the Department and research participation in development and product testing are not enough. Our current and past research program has made it possible to protect crops and livestock against many pests with safety to those crops and livestock, to the user, and to the public from immediate injury. But in our response to the many urgent problems which had to be solved quickly with limited funds and facilities, we have lagged gravely in basic research.

The record of safety in use of agricultural chemicals based on our limited knowledge is amazingly good. I know of no proven case of injury to human health from use of such chemicals according to procedures permitted by the U. S. Department of Agriculture and the U. S. Department of Health, Education, and Welfare; and instances of injury to crops, livestock, and wildlife are small relative to the enormous benefits realized.

We are handicapped in use of existing chemicals and deterred from the use of many new ones by lack of adequate information. There is in current use a biological test so

sensitive it is said to respond to 2 parts per billion of diethylstilbestrol, and I have no doubt that under appropriate conditions it does. Since it is a test for estrogenic activity, would it respond to an equivalent amount of coumestrol, an estrogen which naturally occurs in varying amounts in alfalfa, in an animal grazing on alfalfa or fed on alfalfa meal containing this estrogen? Conversely, would it reveal bound stilbestrol or metabolites of stilbestrol which might be devoid of estrogenic activity but biologically active in other ways?

The use of heptachlor has been severely limited by the discovery that its use under some conditions resulted in small residues of its epoxide. Until we know the metabolic fate of every chemical we use and of all of their metabolites, how can we be sure that we can meet zero tolerances? Until we have quick, specific, reliable, as well as sensitive methods for analysis for each of these chemicals and their metabolites, how can we be sure that prohibited uses have not resulted in illegal residues? Under the Department's meat and poultry inspection responsibility for individual carcass certification for wholesomeness, the urgency for development of such methods is very great.

My remarks so far have dealt principally with an immediate situation. Responsibility for the future is far more important. If our population continues to grow at its present rate, food production must keep pace. At present yields and appetites we can expect shortages within 20 years.

Weeds, disease, parasites, insects, and other hazards presently limit our yields of crops and livestock by an amount estimated to be equal to present yields from 120 million cropland acres--about a third of our present harvest. In addition to demonstrated yield increases from use of chemical fertilizers, success in growth-promotant use of gibberellins, hormones, antibiotics, and arsenicals gives us assurance that research can further increase productive efficiency through appropriate use of chemicals.

Research to meet these needs must be founded on basic research to find specific substances affecting specific metabolic processes specific to certain kinds of plants, animals, insects, or microbes. While we seek such specific information, we must determine any indirect effects.

We all recognize the need for basic research. It has been defined in many ways. My definition is: Basic research consists of the identification and evaluation of parameters, both constants and variables, and the determination of their action and interaction throughout their effective range. Applying this definition to our present need, we must seek, for example, the presence or absence of specific enzymes, such as that which makes the herbicide 2-4-DB a potentially useful mustard killer. We must seek chemicals which will hasten and heighten specific desirable antibody responses. Above all else we must speed up our research on the metabolism of crop plants and livestock species, on their parasites, predators and competing species and on the metabolic interrelationships which determine specific host-parasite and predator-prey relationships. In order to attack these relationships selectively we must know completely the chemical, genetic, and nutritional and ecological factors affecting these relationships.

Let us examine briefly research responsibility in the feed, fertilizer, and chemical application areas. As background for this discussion consider these facts: The only general Federal statute providing regulation of interstate commerce in feed is the Food and Drug Act, which prohibits the movement of adulterated or misbranded feeds in interstate commerce. The New Drug provision of the Food, Drug and Cosmetic Act, the several amendments providing for establishment of tolerances for pesticide and other chemicals, the food additives amendment, and finally the Delaney proviso in the food additives amendment have, in fact, provided a legal basis for Federal regulation of every feed and food moving in interstate commerce, and of fertilizers formulated with pesticides which may be translocated by crop plants and thus enter animal feeds. Veterinary drugs applied by qualified veterinarians and chemicals applied in official regulatory programs are equally affected if such use results in presence of residues of the chemicals or their metabolites in feed or food moving in interstate commerce. It is not enough to have

adequate research information on the safety of a chemical in itself. We must develop adequate information on the formulations used, under the conditions of use. If a coccidiostat in a broiler mash has been demonstrated to result in no detectable residue, will this also be true when a high level of antibiotic is added to the same feed?

Because milk comprises more than a fourth of the weight of food eaten and a far higher proportion of that of infants and of many special diets, no tolerances for residues have been established in milk. This policy poses three research problems for this Department. First, the verification that proposed pesticide and other chemical uses will or will not result in residues in milk. Since it is not possible to prove absence of residue but only presence, reliable, sensitive, quick analytic methods are of utmost importance.

Second, we need more research to develop pesticide chemicals for use on feed crops, dairy animals and premises, which will leave no residue in milk.

Third, in areas where use of pesticides, desiccants, defoliants, and other chemicals is general and especially in areas where aerial application is general, we urgently need research on more precise application to avoid inadvertent residues in milk as well as more effective use of the chemicals.

The breadth of the Department's research interest in agricultural chemicals is the total span of the Department's research activities. No commodity, no functional area, no resource area, no economic or marketing area is untouched directly or indirectly by the efficacy and safety of these chemicals.

The U. S. Department of Agriculture has research responsibility for the development of recommendations for chemical uses which will be safe, effective, economical, and reliable for farmers, for the public, and for regulatory agencies. A severalfold expansion in basic research on the metabolism of crop and livestock species and of their parasites, predators, and competitors is necessary. Special emphasis must be placed on analytic methods, first, to establish conformity with established tolerances with permitted use and, of equal importance, to establish incontrovertibly the presence of illegal residues resulting from inadvertent contamination or illegal use.

REGULATORY AND CONTROL PROGRAMS ON USE OF CHEMICALS

W. L. Popham[1]

The subject assigned to me was "Responsibility of the Department of Agriculture For Use of Chemicals in Agriculture." It is agriculture's responsibility to see that the people of this country derive maximum benefit from agricultural chemicals. To do this, of course, they must be used safely. Chemicals are an essential production tool. They proved our "frontier" in time of most urgent need. If we are to continue producing the food we need, in the variety and quality we want, we have no immediate substitute for chemicals. In my book we have the competence to deal with them in such a way as to derive full benefit without harm to anyone. We have no alternative but to recognize that chemicals are essential to our well-being, can be used safely, and offer our most promising answer to adequate food for the future.

As Dr. Byerly has pointed out, both the Department of Agriculture and the Department of Health, Education, and Welfare have certain functions to perform in assuring a

[1]Deputy Administrator, Agricultural Research Service, U. S. Department of Agriculture.

safe and wholesome food supply. USDA responsibilities are set forth in a series of statutes assigning specific regulatory functions to the Department of Agriculture. These are:

1. The Meat Inspection Act of 1906.
2. The Insecticide Act of 1910.
3. The Agricultural Marketing Act of 1946.
4. The Federal Insecticide, Fungicide, and Rodenticide Act of 1947.
5. The Poultry Products Inspection Act of 1957.
6. Public Law 86-139 of 1959, which extends coverage of the Federal Insecticide, Fungicide, and Rodenticide Act to include nematocides, defoliants, desiccants, and plant-growth regulators.

In addition to these laws, which involve only the Department of Agriculture, the Department was assigned certain responsibilities under Public Law 83-518, commonly referred to as the Miller Amendment to the Food and Drug Act. This Act provides for the establishment, by the Food and Drug Administration, of safe tolerances for pesticides that may leave residues in or on raw agricultural commodities at time of marketing.

In recent years the trend in the Congress has been toward prescribing more pre-clearance of chemicals in dealing with possible residues or other forms of food contamination. This is good--it serves two purposes. (1) It gives greater assurance of safety to the consumer. (2) It gives food producers, processors, and distributors more precise guidelines for assuring the safety of their products.

Being a regulatory man I can't pass up this opportunity to rationalize our aims and objectives with the spirit of the times.

Approximately 50 percent of the more destructive agricultural pests in this country are of foreign origin. Among them are: The European corn borer, the boll weevil, Mexican bean beetle, gypsy moth, and the Japanese beetle. Those who generalize about the balance of nature please take note--if it hadn't been for the carelessness of man these and many other pests and diseases that plague us today wouldn't be here to contribute to the imbalance we hear so much about. Of the 100 most destructive pests introduced in the last 50 years, only about 15 percent have become established in the last 25 years. This record is good. It must be improved--and will be--as our port-of-entry work is strengthened, and the cooperative Federal-State insect and plant and animal disease surveys become more fully operative. Once a new pest of destructive potential--or a disease of livestock--becomes established in this country, we have no practical choice but to contain and eradicate it if possible. Preventive medicine is good medicine.

In the last 30 years we have eradicated the Parlatoria date scale, citrus canker, the citrus blackfly, the Hall scale, the Texas fever tick, and the Mediterranean fruitfly on two different occasions. Insect-borne diseases such as malaria and typhus are no longer a major problem because we have successfully controlled the insects that transmit them.

Our joint efforts with the State of New York have confined the golden nematode, one of the world's most destructive pests of Irish potatoes, to a small area on Long Island for a period of 15 years or more. During this time we have been constantly pressing for a treatment that would achieve eradication. Such a treatment appears to be close at hand.

The khapra beetle has reached the search and treat stage. Of the 709 infestations found as of March 31, totaling more than 169 million cubic feet of storage space, only 14--in California and Arizona--representing some 2 million cubic feet, remain to be fumigated. Additional infestations will undoubtedly be found; however, eventual eradication now seems assured.

The spread of the gypsy moth has been stopped. Infestations once established in Michigan, Ohio, Pennsylvania, and New Jersey no longer exist or will be treated this year.

The white-fringed beetle infests less acreage now than at any time in the last 20 years. Infestations in New Jersey and South Carolina have been eliminated. Substantial progress is being made elsewhere.

No cases of the screwworm of livestock have been found in the southeastern part of the United States since the middle of February 1959. Normally tens of thousands of cases would have occurred since then.

Those close to the problem feel that the witchweed can be stopped and eventually eradicated.

The Mexican bean beetle, a most destructive and difficult pest to control, became established in California in 1946. Nine years later, and at a cost of some $2-1/2 million, it had been eradicated at State and county expense.

By the intelligent use of interstate quarantines, West Coast States have been kept free of such pests as the Japanese beetle, corn borer, apple maggot, and the plum curculio, to name only a few.

Short of eradication, continued and persistent effort has slowed the spread of the pink bollworm, the Japanese beetle, and more recently, the imported fire ant.

Ways have been found to confine the sweetpotato weevil, the phony peach disease, and peach mosaic to a small part of the area they would normally occupy.

The Mexican fruitfly has been kept out of California and Florida, and from becoming a major consideration in the production of citrus in the lower Rio Grande Valley of Texas.

We now have procedures for preventing catastrophic outbreaks of grasshoppers which, in a number of instances in years past, have prompted joint resolutions in Congress and in State Legislatures.

Outbreaks of the foot-and-mouth disease have been eradicated on nine different occasions, thus protecting both our livestock and game animals from a most costly and destructive disease. Vesicular exanthema has been eliminated after spreading to 42 States. And now the Southeast has been freed of the screwworm.

It is impossible to interpret in dollars and cents what the control or eradication of the malarial mosquito, the Medfly, the Texas fever tick, and the screwworm has meant to agriculture and to the general economy of the South.

A major factor in this progress has been the availability of new and effective chemicals combined with positive thinking and action. Had we waited for all the answers before undertaking these programs, we would still be waiting. We learn as we progress--we have had to be alert to pitfalls, and to the opportunities for modifying program procedures as new information became available. A constant review of operational procedures is essential.

I need not belabor you further about the place chemicals occupy in agriculture.

In many of his operations a farmer can work independently--what his neighbor does has little effect on what happens to his crop--but one farmer can not harbor an infestation of pink bollworm, the screwworm, or Mediterranean fruitfly without creating a situation of profound concern to his neighbors. Foot-and-mouth disease, brucellosis, bovine TB, vesicular exanthema are not problems to be dealt with on an individual basis. Farmers are coming to recognize this and have turned their thinking more and more in the direction of animal disease and pest control on an area basis. This is a healthy trend--one to be encouraged.

In my opinion any program that will eliminate from this country, or even stop the further spread, of an introduced pest or disease should have the outspoken support of both conservationists and regulatory agencies concerned with the wholesomeness of our food supply.

If we have no pests--we need no pesticides.

Gentlemen, let's tune out the static and get to work. This residue problem is a big one--it's complex--but it can be solved.

PESTICIDE RESIDUES IN RELATION TO THE FOOD, DRUG, AND COSMETIC ACT

Robert S. Roe[1]

This first session of the symposium deals with responsibilities--the responsibilities of the Department of Agriculture, those of the Food and Drug Administration, and those of industry. This seems an appropriate note on which to begin this presentation and discussion of chemicals used in agriculture. It implies that the government and industry groups concerned have an accountability for some phases of the uses of chemicals in agriculture. This implication is well founded. The large number of chemicals in use today, the considerable quantities used, the potency of many of them, and the potentiality for harm when misused certainly call for and require the exercise of a high degree of responsibility.

The Food and Drug Administration has no direct responsibility in determining or developing the agricultural uses of chemicals. Nor is it responsible for regulating the chemicals or the commercial formulations containing them, except in the case of those that fall in the category of drugs or food additives. The Food and Drug Administration is responsible for administering the Food, Drug, and Cosmetic Act, a principal purpose of which is to insure the integrity and safety of foods distributed in interstate commerce. Agricultural uses of chemicals that leave residues in food products derived from treated crops or animals are of concern to the Food and Drug Administration. It becomes a responsibility of the Food and Drug Administration to establish regulations including safe tolerances for residues in such foods.

I should like to review the responsibilities of the Food and Drug Administration under the Food, Drug, and Cosmetic Act that directly or indirectly deal with chemicals used in agriculture. There first comes to mind, of course, pesticide chemicals--insecticides to prevent or control insect damage to crops; fungicides to prevent plant disease or fungus damage; herbicides to control weeds; and fumigants for protection of grains. And more recently, this category includes plant growth regulators, sprout inhibitors, defoliants. Chemicals added to animal feeds or otherwise applied to animals as fattening agents or growth promoters may be of concern under the recent Food Additives Amendment. And then there are many veterinary drug preparations used to prevent or treat diseases, some of them antibiotic preparations subject to certification by the Food and Drug Administration for safety and efficacy, that come within the responsibility of the Food and Drug Administration.

The Miller Amendment (or Pesticide Chemicals Amendment) enacted in 1954 provides a procedure for setting safe tolerances for residues of pesticide chemicals in raw agricultural products or for the establishment of exemptions from the necessity of

[1]Director, Bureau of Biological and Physical Sciences, Food and Drug Administration, U. S. Department of Health, Education, and Welfare.

tolerances when such are not necessary to protect the public health. For the purposes of this law the term "pesticide chemical" means any substance which is an "economic poison" under the Federal Insecticide, Fungicide, and Rodenticide Act and is used in the production, storage, or transportation of raw agricultural commodities. Any person who has submitted an application for the registration of an economic poison under the Federal Insecticide, Fungicide, and Rodenticide Act in the Department of Agriculture may file with the Department of Health, Education, and Welfare a petition proposing a tolerance or an exemption. The petition must be supported by certain information including the identity and composition of the pesticide chemical, methods for analysis, complete information on the use, full reports of investigations made with respect to the residues produced and, of course, information on the toxicity of the chemical. The applicant also submits a copy of the petition to the Department of Agriculture and when the Secretary of Agriculture issues a Certificate of Usefulness, then the Secretary of Health, Education, and Welfare has 90 days to act upon the petition and issue a regulation. He may set the tolerance at zero if the data do not justify a greater tolerance.

Provision is made for referral of the petition to an advisory committee if either the petitioner or the Secretary desires such referral within the 90-day period. After publication of an order, any person adversely affected may file objections and request a public hearing. Finally, provision is made for court review under certain circumstances involving an actual controversy.

The technical review and evaluation of petition protocols are the responsibility of the scientific staff. This is a most serious and important responsibility, which can only be discharged adequately by well-trained and experienced scientists.

In establishing a tolerance regulation, the law admonishes that, among other relevant factors, appropriate consideration should be given to the other ways in which the consumer may be affected by the same pesticide chemical or by other related substances that are poisonous or deleterious.

In appraising petitions our first concern is to determine if the tolerance proposed is, in fact, a safe level. Next, we ask whether the tolerance level sought is necessary for effective use of the pesticide chemical on the particular crop or crops. We think that the tolerance should not be set at any higher figure than is necessary even though a higher figure would be safe. We believe that this policy is sound and is wholly consistent with the intent of the law.

The problems in reviewing petition data include consideration of the chemistry of the pesticide: Are we certain of its composition and chemical identity? Are we sure that the residue present at harvesttime is the same compound that was applied to the crop--or have there been chemical changes--oxidation, for instance? Is the substance systemic--does it translocate into the tissues of the plant? If so, are there metabolites? Are the methods of analysis adequate to detect and measure residues that may be present? Will the methods detect oxidation products or metabolites? Do the data adequately validate the methods employed in securing the residue data?

And then, a method may be entirely suitable for measuring residues remaining from test applications under controlled conditions. But we must consider the adequacy of methods in terms of their ability to detect and measure the residues in question in the presence of possible interfering substances. Unless we have methods capable of and practicable for enforcing tolerances, such tolerances can hardly be regarded as safe. Accordingly we are including an appraisal of methods of analysis as a part of our review of petitions. This may include actual try-out of the methods in our field laboratories.

A very important part of the petition review involves the evaluation of the toxicity tests. This requires a meticulous study of the detailed data and an appraisal of the appropriateness of the experimental design and the validity of the conclusions. Are we satisfied that the toxicity data relate to the residues in question?

Over 2,000 tolerances have been established, considering the different levels and the various crops, involving over 100 pesticide chemicals.

The Food Additive Amendment enacted in September 1958 became fully effective on March 6, 1960. The term "food additive" means: Any substance the intended use of which results--or may be expected to result--in its becoming a component or otherwise affecting the characteristics of any food--
IF such substance is not generally recognized by experts qualified by scientific training and experience as having been shown through scientific procedures to be safe (or re substances used prior to January 1, 1958, through either scientific procedures or experience based upon common use in food).

It does not include pesticide chemicals on raw agricultural commodities (they are regulated under the Pesticide Amendment) nor does it include substances which enjoy prior sanctions under the Food, Drug, and Cosmetic Act, Poultry Products Inspection Act, and Meat Inspection Act.

The term "food additive" does, however, include any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding, and, any source of radiation intended for any such use.

Uses of chemicals in the treatment of manufactured or semimanufactured agricultural products--such as dried fruits; prepared feeds; packaging materials for dairy products; applications to food animals that might place residues in food products--are examples of applications in agriculture that may call for regulations under the Food Additive Amendment.

The procedure for obtaining a Food Additive regulation is similar to that for a pesticide tolerance. There is, however, no provision for review by an advisory committee and the time for evaluation of a proposal can be extended an additional 90 days, if necessary. The amendment contains these significant directives: If a tolerance is required to assure safety:

1. It shall not be at a level higher than reasonably required to accomplish the physical or other technical effect intended; and

2. Regulation shall not be established if data do not establish that the use would accomplish intended effect.

No regulation shall issue if the data:

1. Fail to show that proposed use will be safe

   Proviso--(Delaney clause) No additive is deemed safe if found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal.

2. Show that the proposed use would promote deception of consumers or otherwise result in adulteration or misbranding under this Act.

There are two other sections of the Food, Drug, and Cosmetic Act on which I wish to make brief comment. One deals with antibiotics that are subject to certification, that is penicillin, streptomycin, chlortetracycline, chloramphenicol, and bacitracin, and derivatives.

Batches of drugs containing these antibiotics are subject to certification. The regulations established under this provision of the law are intended to insure the safety and efficacy of use of these drugs. Many veterinary preparations for use in farm animals have been the subject of antibiotic regulations or exemptions.

The New Drug section of the law requires that new drugs must be the subject of applications containing information adequate to establish the safety of the drugs for the intended uses. Here too, many veterinary preparations are involved.

In evaluating the safety of certifiable antibiotics and new drugs intended for veterinary use, we take into account, not only the safety to treated animals, but the safety of any residues that may occur in the food products derived from the animals. The term "drug" as used in the Act includes substances intended to affect the structure or any function of the body of man or animal as well as those intended to treat or prevent disease. Hence, substances added to feeds as fattening agents or growth promoters are drugs. If they place residues in the food products--meat, milk, eggs--they may also be food additives. Many of these types of preparations require consideration as food additives as well as new drugs or certifiable antibiotics.

It is, then, the responsibility of the Food and Drug Administration to establish regulations under the applicable sections of the Food, Drug, and Cosmetic Act calculated to insure the safety of our food supply. This responsibility does have some impact on the use of chemicals in agriculture.

ROLE OF INDUSTRY IN DEVELOPMENT AND USE OF AGRICULTURAL CHEMICALS

George R. Ferguson [1]

On behalf of the National Agricultural Chemicals Association, I would first like to extend the thanks and appreciation of our industry for the opportunity afforded here to present some views that might not otherwise be taken into account in the deliberations of this symposium. I would also like to state that the writer (speaker) takes full personal responsibility for the views presented as I have not limited my remarks to matters of established policies of the Association or of my own Company.

The use of the term "responsibility of industry" in the assigned title is extremely broad in scope and perhaps could be construed to cover such subjects as profit margins and "payola" as developed in recent Congressional Hearings. Fortunately, these subjects are outside the scope of this symposium. Furthermore, our segment of the chemical industry is notorious for slim profit margins and highly competitive practices which have resulted in a number of firms going out of the pesticide business in recent years. Trends toward increasingly restrictive legislation and regulation at local, State, and Federal levels of Government are accelerating this outward movement as more and more management groups decide that money can better be invested and the stockholders better protected in other types of business.

Although I will comment only with regard to responsibilities of the chemical industry, I cannot help but note that other segments of industry, such as the foodpacking and processing industry, the farm equipment industry, and perhaps others have a major stake in any problem involving agriculture.

More important is the overall responsibility of agribusiness as a whole. Out of some $33 billion of off-farm sales of food and fiber, some $15 billion represents farmer purchases of seed, fertilizer, fuel, pesticides, and other farm supplies. The value of this food and fiber at the consumer level is estimated at over $90 billion. I submit that the industries responsible for upgrading $33 billion of farm products to $90 billion of consumer products also have a stake and responsibility in the problems to be discussed here.

---

[1] President, Geigy Agricultural Chemicals, and Vice-President, National Agricultural Chemicals Association.

As an additional background factor, it should be noted that it has been established Government policy for many years to encourage a high level of agricultural production through price supports and research, even at the expense to the taxpayer of the cost of storing and disposing of huge surpluses. Production has been controlled through acreage allotments, soil bank incentives, and similar measures rather than limitations on yield per acre or quality.

Granted that this program has created problems of a highly controversial nature, both politically and economically, it has, nevertheless, been a reasonably consistent policy. We have every reason to assume that the Congress of the United States will continue to maintain some type of support program, especially as our population growth narrows the gap between food production and consumption.

Minimum prices at around support levels under conditions of surplus production provide a tremendous stimulus to the farmer to reduce unit costs. Unit costs are reduced through greater yields per acre, reduction in labor utilization, and larger sized production units. Obviously the result must be rapid mechanization and chemicalization in agriculture as well as a move toward larger and larger farming units under the pressure of mass production methods. In 1954, only 10 percent of our farmers produced almost half of our off-farm sales of food and fiber. There is little question but what this picture will be further changed in the 1960 census.

The use of pesticide chemicals, therefore, is not only a necessity from the standpoint of pest epidemics and direct crop losses, but also an economic necessity from the standpoint of maintaining quality, lowering unit production cost, and in offsetting a decreasing farm labor supply. We have always before us the possibility that some growers, who are on the verge of going out of business, owing to lack of labor for hoeing weeds or some other factor, may misuse pesticidal chemicals as a last desperate gamble because they have everything to gain and nothing to lose. A drowning man is often not too concerned whether or not he pulls someone else down with him even though on dry land he may be the most responsible and public-spirited man in his community. I do not feel that these economic pressures in our farming industry and their causes have been sufficiently taken into consideration in the many deliberations on the problems involved in chemical residues.

The responsibilities of the chemical industry in this whole field of pesticide residues are difficult of definition. The chemical industry is reevaluating its position as well as its responsibilities. Some large chemical corporations have either cut back or abandoned research in this field and others are considering following suit. It becomes difficult to count beyond 12 large chemical concerns worldwide who are carrying on basic pesticide research worthy of the name. Basic research by the smaller chemical companies is virtually nonexistent.

Many promising chemicals have been dropped from development programs because potential markets have not been large enough to justify development expense. It has been suggested in some quarters that industry is shirking its responsibilities by not developing promising products even though they will lose money. This assumes that every company has some inexhaustible pot-of-gold that allows it to forget about making a profit. Industry has a responsibility to its stockholders as well as to the public and to its customers. As long as we have a free-enterprise system, this will remain so, whether it be chemicals, farm products, or airplanes. We can establish, therefore, one basic responsibility of industry, namely, to make a profit as justification for the stockholder's investment in the enterprise. The weekly index of business failures published by the Department of Commerce is testimony to the fact that all businesses are not necessarily successful.

Let us look more specifically at the pesticide field. Agricultural pesticide sales at the farmer level represent only about 2 percent of total chemical industry sales--about $500 million out of a $25 billion industry. It should be apparent that financial management is reluctant to take large risks in a field that represents only 2 percent of the industry. These risks include: (1) High investment in development and plant, (2) problems

in obtaining adequate product liability insurance, (3) product obsolescence through insect resistance or other factors, and (4) possible rescinding of tolerances once established.

Industry today, by and large, has a keen sense of public responsibility. The company reputation and the "corporate image" are intangibles on which many millions of dollars of institutional advertising and public relations have been spent. Some firms do not want to take any risk whatsoever in a controversial field where the "corporate image" may suffer, and would prefer to forego business in a relatively small market such as ours than to have aspersions and innuendo cast by even such groups as the nature fanatics or food faddists.

Although the above may represent a minority viewpoint, the industry view of public responsibility is a very real thing. Our industry both through individual company effort and through our Association has devoted a large part of its public relations effort toward explaining the need for chemicals in agriculture and public health, and in emphasizing that they can be used safely when directions are properly followed. I do not believe any major company today would in any way deliberately jeopardize its public relations by marketing a product for which it was not satisfied as to its safety and utility in its intended use.

It is doubtful in my opinion, however, that safety requirements can be legislated. Evaluation of safety is a constantly changing thing as new products and new data become available. It requires expert scientific judgment--not legislation. There is no question but what conservatism must prevail in any approach to the problem of public exposure to chemicals in foods, but the "rule of reason" must be used in arriving at conclusions within the framework of existing scientific knowledge.

Although we pride ourselves on our advanced state of agricultural technology, it is worthy of note that the farmer in Western Europe has more chemicals available in his pesticide arsenal than the United States farmer. I am in no position to know whether any real hazards exist to the European population as a result, but the United Nations has reported that at least some European countries have better average dietary and health standards than the United States average even with all of our food surpluses. I do not imply in any way any cause and effect relationship, but only that Western European governments are also conscious of the need for maintaining a safe and wholesome food supply and in protecting the health of their publics.

During the past decade a disproportionately large percentage of new pesticides have originated from European research laboratories which may indicate that lower development costs serve as a better stimulus to pesticide research overseas than in the United States.

Perhaps the most important industry responsibility is to its customers and users of its products. This responsibility carries many broad implications. Our products are sold and purchased in the good faith that they will perform as claimed when directions are followed, that the user will not damage his crop nor harm himself in its application, and that the resulting crops can be sold in interstate commerce without restriction or violation of any State or Federal statute.

In order to properly advise the users of our products, we must know what we are selling, its biological characteristics, its toxicology, what residues may result, the fate of these residues, and how to measure them. Industry fully accepts the responsibility for developing this information on its products even though disagreements as to scope, extent, and adequacy of the data are frequently encountered between industry representatives and regulatory agencies. Under existing legislation, we are faced with problems of negative proofs, such as proof of safety and proof of zero. These present problems of methodology, techniques, and individual interpretation that could carry on such studies for many years. It has been suggested recently that 7-year dog feeding studies may be required when a chemical is suspect of having carcinogenic activity.

There is no question but that our problems are becoming more complex, if for no other reason than our chemicals have become more complex. Most of the simple chemicals have been tested and evaluated. In order to evaluate the newer and more complex synthetic organic chemicals, we must without question proceed to a higher plateau of technology. This is true in our economy today whether it be chemicals, jet engines, or atomic energy.

However, if unnecessary restrictions force free enterprise out of the pesticide field, then the development responsibility undoubtedly will fall on Government agencies, and we will be one more step on the way to socialization. Pesticides are one of the essential links in the chain of food production. It is, of course, axiomatic that a chain is only as strong as its weakest link.

When Premier Khrushchev visited this country recently, he threw down the gauntlet of challenge to our free enterprise system in the field of agricultural technology and production. Since the chemicals available in Western Europe are presumably available also in Eastern Europe and Russia, it could be argued that the Russian collectivized farm or "commune" is already ahead of our farmers technologically in the pesticide field. I am not implying in any way that we should relax any safeguards required to protect the health of the American public; however, I am saying that overly restrictive or punitive legislation and regulations can very definitely put American agriculture at a technological disadvantage compared to agriculture behind the Iron Curtain.

Any reduction in agricultural research today, whether in industry or government, can well have an adverse effect 10 to 20 years from now when our population and food supply will more nearly be in balance. The chemical industry wants to stay in this field, is willing to spend huge sums on research and development, and is willing to carry its full share of responsibility. All we ask for is: (1) More emphasis by governmental and institutional agencies on basic biological research in such fields as plant and animal physiology, basic biology, and most important--biochemistry; and (2) some assurances that the approach to the problems of protecting the public health and the consumer will be based on sound scientific fact.

In conclusion, I can do no better than to quote Dean Earl L. Butz of Purdue University who, in a recent talk [2], concluded with:

> "We must search diligently for a basis of common understanding among government regulators, research scientists and business executives so that we can meet the dual objectives of a safe and healthful food supply on the one hand, while at the same time availing ourselves of every scientific aid to produce, process and merchandise that food supply ever more efficiently and economically.
>
> "These dual objectives are not in conflict. They are completely compatible and attainable if sensible and determined men can set themselves diligently to the task in an atmosphere of scientific objectivity, unimpeded by such modern phenomena as the news release, the press conference and the congressional hearing."

It is of special interest to the chemical industry that this symposium has been organized in such "an atmosphere of scientific objectivity," and it is to be hoped that the end result will be even greater emphasis by Government scientists on research in this very basic field of pesticide residues.

---

[2] Butz, Earl L., Croplife, pages 7-8, April 4, 1960.

AGRICULTURAL CHEMICALS IN RELATION TO HUMAN HEALTH[1]

Wayland J. Hayes, Jr.[2]

(Abstract)

Pesticides promote health directly through the control of vector-borne diseases and indirectly through increased and improved agricultural production. On the contrary, the extensive use of any biologically active chemical implies a potential hazard which has to be evaluated. Production and use of these materials have increased greatly since World War II. In the United States alone, the annual production of synthetic organic pesticides, which were essentially unknown before DDT, reached a level of 569,927,000 pounds in 1956. While this production of these synthetic materials has increased from essentially zero in 1942, production of the older, largely inorganic, pesticides has shown only a partial decline. Although something less than 200 basic chemicals are really important as pesticides, the situation is complicated for the practicing physician by the fact that thousands of different formulations of them may be labeled and sold in a single country. The situation is further complicated by the fact that pesticides are now applied in ways which were either unknown or uncommon a few years ago. An example is the extensive use of aircraft for spraying operations.

The way in which a compound is used may contribute more to its danger than its inherent toxicity contributes. Extensive experience has shown that pesticides may be used with very few accidents provided scrupulous attention is paid to their labeling and use. The death rate from poisoning caused by all pesticides in the United States is slightly less than 0.09 per hundred thousand population. Strangely enough, most of the fatal accidents in this country still involve the older pesticides. For example, in 1956, there were a total of 152 accidental deaths caused by all pesticides. Of these, 104 were caused by compounds older than DDT, including 54 caused by arsenicals alone. Over half of the cases occur in children. Thus in 1956, 94 of the 152 cases were in children under 10 years of age and 78 were in children under 3. Both the kinds of compounds involved and the predominance of cases in children indicate the possibility of real improvement if people can be educated to use these materials with even greater care. The safety record in some other countries is not so favorable, especially in connection with the newer compounds. It appears likely that improved labeling and improved regulation of use in those countries would help to remedy the situation.

Information on morbidity is far less precise than that on mortality. In one study in which only serious cases were counted, the ratio of fatal to nonfatal cases associated with poisons was 1:13. In another study in which every report of trouble was counted as a case, the ratio was 1:750. The ratio is commonly estimated as 1:100 and this comes as close to the truth as is possible at this time. One gets the impression that the newer pesticides cause more nonfatal accidents than the older compounds cause. This is what one would expect from the amount of the various compounds used. However, the conclusion must be taken with reservation because reports of fatal poisoning by pesticides in the lay and technical press are chiefly concerned with the newer compounds and yet the official statistics show that in this country the majority of cases are caused by the older compounds.

---

[1] From the Technical Development Laboratories, Technology Branch, Communicable Disease Center, Bureau of State Services, Publication Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Ga.

A review by the speaker entitled "Pesticides in Relation to Public Health," Ann. Rev. Entom. 5: 379-404, 1960. Annual Reviews, Inc., Palo Alto, Calif. A review by J. M. Barnes entitled "Toxicity of Pesticides," Bul. Hygiene 34: 1205-1219. December 1959.

[2] Medical Director and Chief, Toxicologist, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Ga.

By necessity, first estimates of the safety of a pesticide must be based on its toxicity in experimental animals. Such study may provide essentially all the information that is necessary in evaluating the probable results of acute exposure such as that involved in accidentally eating the compound. However, additional studies must be made if the mode of action of the compound is to be understood and the danger of occupational poisoning is to be evaluated. The study of dermal toxicity in animals is more useful than the study of oral toxicity in predicting occupational hazards. However, there are sufficient differences in the reactions of different species to the same compound that studies must eventually be made in man himself. Such studies, in man, have been made of a number of compounds including DDT, schradan, dimefox, parathion, Dipterex, malathion, tetraethyl pyrophosphate, and dinitrophenol.

It may be nearly as valuable to know the magnitude of exposure by different routes as to know the toxicity of a compound. It has been known for some time, on the basis of animal experiments, that many of the newer pesticides, except DDT, are absorbed easily through the skin, even when not in solution. This property has been associated correctly with cases of poisoning following massive skin exposure, but the importance of dermal absorption in the more ordinary cases of occupational illness frequently has been missed. Actual chemical measurement of the exposure of applicators to several compounds used in agriculture or in vector control has shown that skin exposure is greater and sometimes very much greater than respiratory exposure when liquid sprays are used. However, not all work situations have been explored and additional studies need to be made. It has been necessary to develop new techniques for measuring the exposure to pesticides because the older methods used for measuring exposure in factories and mines proved inadequate. The newer methods are useful not only in evaluating different routes of exposure, but may be used also in studying the efficiency of protective clothing and devices.

Incidental or environmental exposure to pesticides has been a source of difficulty only rarely, as for example, the allergic reactions of a few people exposed to vaporized lindane. Excluding acute poisoning following massive exposure, any ill effects are to be expected in persons whose exposure is intensive and prolonged. A study of such people is also the best single source of information about the potential long-term hazard to the general population in the use of any particular compound.

Nothing is now known to justify the suggestion from some sources that pesticides currently constitute a serious threat to human health and that they are now a significant cause of blood dyscrasias and other diseases. However, it is the duty of the Public Health Service to keep alert to such possibilities. In addition to our own planned studies, we welcome reports of cases or any other relevant information.

There is now a considerable amount of information about the clinical effects of poisoning by a wide range of pesticides in man. At least 48 of these compounds are known to have produced human poisoning. Some useful information on the treatment of poisoning by many of them is available. However, much remains to be learned about symptomatology and treatment, as well as about the prevention of poisoning.

In conclusion, the human safety record of the newer compounds is good in some technically advanced countries, largely owing to careful labeling. In countries where labeling is poor, or where illiteracy or irresponsibility tend to vitiate labeling, hundreds of cases of human poisoning have occurred. Because of the usefulness of economic poisons in the control of vector-borne diseases and also in the control of a wide range of pests of field crops, livestock, forests, and stored products, it should be our aim to improve their safety so that they may be fully exploited.

SECTION II--PROGRESS AND PROBLEMS

USE OF HERBICIDES GROWTH REGULATORS, NEMATOCIDES, AND FUNGICIDES

W. B. Ennis, Jr.[1]

INTRODUCTION

Remarkable progress was made during the last two decades in developing and in using herbicides, growth regulators, nematocides, and fungicides to improve the efficiency of agricultural production. The decreasing labor supply and the steadily increasing necessity for mechanization of agriculture require more and more chemicalization of agricultural production. The progress made, the materials and "know-how" available, and the continuing search for new and improved chemicals give us every right to believe that concentrated basic research will give more effective and safe practical methods of combatting weeds, plant disease organisms, nematodes, and other problems including the regulation of plant growth.

This paper deals primarily with highlights of early and recent progress in using certain noninsecticidal chemicals to solve major pest problems in American agriculture, and presents examples of unsolved problems which require cooperative basic research for solution. Lines of research are outlined, which require increased scientific manpower and support, if the development and safe use of noninsecticidal chemicals are to keep pace with agricultural needs.

EARLY PROGRESS

Fungicides--Fungicides were used earlier than herbicides, nematocides, and growth regulators. As early as 1,000 B. C., Homer spoke of "the pest-averting sulfur" (5), and as early as 1705 mercuric chloride was recommended as a wood preservative (3). In 1761 copper sulfate was used with variable results for the control of bunt on wheat seed (5).

The modern era of fungicide research dates from Millardet's discovery of Bordeaux mixture in the vineyards of France during the severe epiphytotic of downy mildew of grape in the 1880's (3). For the next 75 years it was used increasingly and was a frontline defense against the ravages of the late blight fungus of potatoes. Up to about 1930 Bordeaux mixture, lime-sulfur solution, and self-boiled lime-sulfur were the standard control fungicides (2).

Introduction of organic mercury compounds for seed treatments in 1915 (4) provided a means of breaking the pernicious transmission of plant pathogens from one crop to another by way of seeds.

---

[1]Chief, Crops Protection Research Branch, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

Herbicides--Use of chemicals to control weeds dates back to 1895 to 1909. During that period copper sulfate, common salt, iron sulfate, sulfuric acid, carbolic acid, formaldehyde, sodium arsenite, and sodium arsenate were studied in this country and in Europe for control of weeds. In the 1930's the selective herbicidal actions of certain dinitro compounds were discovered by workers in France. Some of these early explorations demonstrated the feasibility of chemical control of weeds and provided stimulus for continuing search for chemicals for selective killing of weeds among crop plants. Early use of chemicals for weed control was limited, because they lacked high potency and adequate selectivity.

Discovery of selective herbicidal action of phenoxyacetic acids in 1943-1944 is the major breakthrough with far-reaching effects on weed control. Since this discovery a new science of weed control has rapidly evolved.

Nematocides--Although plant parasitic nematodes have been studied for more than a century in Europe the discovery and development of nematocides have been slow because of the small number of workers concerned with nematodes and their control. Although carbon bisulfide was used in Germany in 1881 to kill sugar beet nematodes without notable success, as late as 1911 it was still considered the most efficient chemical for the control of nematodes in the field. Even though effective it has never been used extensively (6).

Of the nematocides used today, chloropicrin was studied first in Great Britain in 1919. Subsequently it was evaluated on a field basis in this country and later in Hawaii. By 1935, chloropicrin was used rather extensively for nematode control in pineapple fields. Thereafter it was sold as a nematocide, largely for use in greenhouses and seedbeds (7).

The successful use of methyl bromide for controlling soil nematodes in 1940-41 (8) provided the first real stimulus for synthesis and development of additional chemicals for controlling nematodes in soils in the field.

Today more than 10 nematocides are produced commercially and their retail sales are estimated as more than $16 million. Since few growers use soil nematocides except where returns from the crops are at least four times the cost of the nematocides, farmers presumably profited to the extent of more than $64 million last year through the use of nematocides. Nematocide application has become a standard practice for producing pineapples in Hawaii and tobacco in many parts of the Southeastern States.

Growth Regulators--The present availability of selective organic chemicals that regulate plant growth and control weeds in crops can be traced to investigations of Darwin who published a book called "The Power of Movement in Plants," in 1880. Work on the naturally occurring plant auxins a and b and the discovery of 3-indoleacetic acid as a plant-growth regulator stimulated attempts to isolate the chemically pure compounds from plants and to synthesize compounds that would influence the growth of plants.

Among the synthetic organic compounds evaluated as growth regulators was 2,4-dichlorophenoxyacetic acid (2,4-D). When tested as a herbicidal agent 2,4-D was found to have extremely high potency in killing broadleaved weeds and strangely did not noticeably harm grass plants. After this discovery of the selective control of weeds with highly potent organic chemicals, many industrial and public agencies took rapid steps to synthesize and evaluate additional organic compounds as growth regulators and selective herbicides.

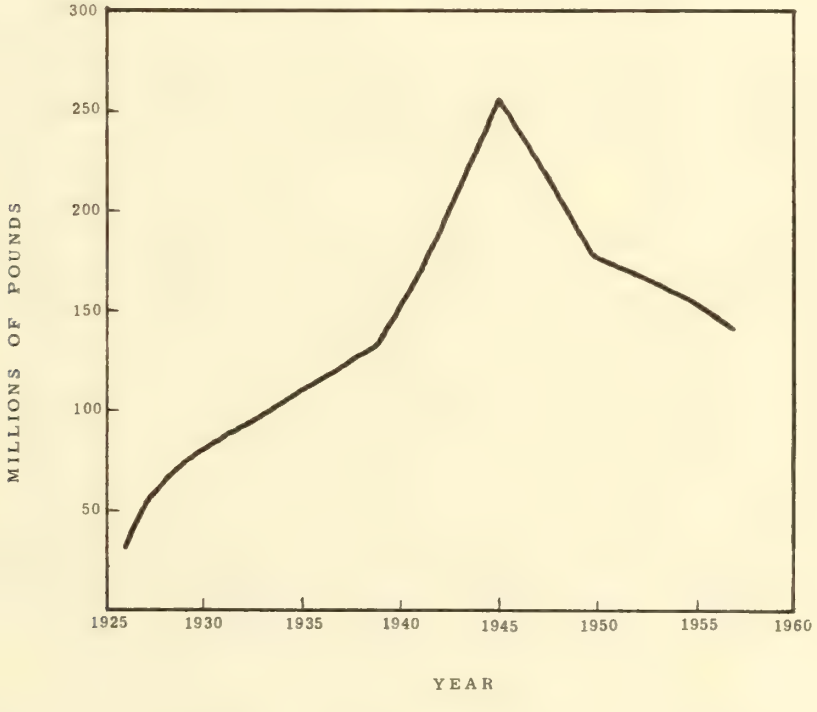
RECENT PROGRESS

The number of noninsecticidal pesticides produced and used in the United States reflects the progress in the development of these materials for agricultural uses (table 1).

TABLE 1.--Approximate number of noninsecticidal pesticides in use in various years, 1800 - 1960

Year	Fungicide		Nematocide	Herbicide		Growth regulator
	Inorganic	Organic	Organic	Inorganic	Organic	Organic
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
1800	2	0	0	0	0	0
1850	2	0	0	0	0	0
1900	4	4	1	5	1	0
1920	7	4	2	6	1	0
1930	9	4	3	8	3	0
1940	9	29	3	8	6	4
1945	9	31	5	9	10	7
1950	9	33	6	9	22	9
1955	9	35	8	10	38	12
1960	9	36	12	10	57	16

Before 1940 protectant fungicides were almost exclusively inorganic. Subsequently, use of inorganic fungicides, with few exceptions, dropped as they were replaced by such organic materials as the dithiocarbamates, quinones, and s-triazines (figure 1). A similar situation developed with herbicides although large tonnages of inorganics such as chlorates, borates, and arsenicals are still used on noncrop areas. Synthesis and evaluation of new herbicides and growth regulators are currently expanding rapidly, but the rate of introduction of additional fungicides appears to have leveled off.



BN-10806-X

Figure 1.--U. S. Production of copper sulfate for fungicides in years, 1926-1957. (From Agricultural Statistics, U. S. Department of Agriculture.)

The availability and increasing use of a sizeable arsenal of fungicides, growth regulators, herbicides, and nematocides have made possible tremendous savings to farmers in improving the efficiency of producing high-quality food, feed, fiber, timber, and ornamental crops.

Herbicides--A recent survey by the Federal Extension Service, in cooperation with the Agricultural Research Service and 48 of our States, shows that about 50 million acres of agricultural land were treated for weed control in 1959 (table 2). The acreage treated with herbicides in 1959 was more than double that in 1949 (table 3). The expansions in the use of chemicals for controlling weeds are further illustrated by data from Minnesota on the acreage of grain crops treated yearly since 1950 (figure 2) and the number of power sprayers used for weed control during the same period (figure 3).

More than 16 million acres of corn were treated in 1959 (table 4) at a cost of about $36 million. Chemical control of weeds in a crop such as corn eliminates two cultivations. Considering that each cultivation costs about $1.50 per acre, the benefits from reduced cultivations alone on these 16 million acres are estimated at $12 million. Additional benefits are reduced weed seed populations, improved picker efficiency, and higher yields due to reduced weed competition for light, moisture, and nutrients.

Equally striking benefits are being obtained from use of herbicides in controlling weeds in (1) pastures and rangelands, (2) drainage and irrigation systems, (3) certain horticultural crops, (4) other agronomic crops, and (5) on numerous noncrop land sites. By applying the net savings per acre realized from the use of herbicides on corn to the 50 million acres of farmlands presently treated with herbicides, it may be conservatively estimated that the net savings to American farmers exceeds $40 million annually. If herbicides were used for weed control on all acreage of cropland and grassland, the annual savings could exceed $1.5 billion annually and could release millions of man-hours for other enterprises.

The opportunities for additional contributions through developments to control weeds on cropland as well as on noncrop land are indeed challenging. Herbicides are used for weed control on utilities, railroad, and highway rights-of-way, and on drainage and irrigation ditches. They afford means of combatting ragweed, poison ivy, and other weeds which cause hayfever and other debilitating allergies.

Growth Regulators--Growth regulators are currently used for a multiplicity of purposes in modifying the growth and development of crop plants. Most commercial plant propagators now use as standard procedure one or more of the root-inducing chemicals. Regulators are also utilized for controlling the harvest drop of fruits, chemical thinning of fruits, increasing rate of fruit development and size, hastening maturation, improving storage quality of vegetables, increasing plant size and rate of growth, reducing plant size, and prolonging plant life. Use of growth regulators for treating orchard and certain other crops has increased gradually from about 10,000 acres treated in 1940 to an estimated 800,000 acres today.

Growth regulators of the defoliant and desiccant type are broadly used to improve grade and quality of machine-harvested cotton and reduce the incidence of boll-rotting diseases. The acreage of cotton treated has increased from about 250,000 acres in 1946 to an estimated 6,250,000 acres for the 1960 growing season (table 5).

Nematocides--Nematocide applications are highly profitable in certain areas to control plant parasitic nematodes in soils to be used for many crops of high value per acre. Many of our best producing areas are so infested with nematodes that high-quality, high-yielding crops such as sugar beets, carrots, pineapples, and tobacco can no longer be grown profitably without the use of nematocides or resorting to rotational or fallowing practices with the consequent loss in profits per acre.

TABLE 2.--Estimated total acres treated for weed control on farms in United States, 1959[1]

Crop or area	Post-emergence	Pre-emergence	Total
Peanuts	3,000	29,130	32,130
Sorghum	2,075,000	8,000	2,083,000
Sugar beets	44,200	78,600	122,800
Small grain	19,475,200	500	19,475,700
Corn	14,224,370	2,198,300	16,422,670
Cotton	278,600	890,600	1,169,200
Soybeans	10,000	518,435	528,435
Rice	637,000	0	637,000
Vegetable crops	204,080	71,865	275,945
Fruits and nuts	5,425	35	5,460
Strawberries	3,300	2,000	5,300
Lawns	63,800	1,250	65,050
Ornamentals	2,240	210	2,450
Pastures	2,212,450	30,100	2,242,550
Rangelands	4,296,000	0	4,296,000
Hay	186,400	0	186,400
Forage seed	261,300	100	261,400
Subtotal	43,982,365	3,829,125	47,811,490
Noncrop land	2,889,500	200	2,889,700
Total	46,871,865	3,829,325	50,701,190

[1] From unpublished survey data accumulated by the Federal Extension Service; Farm Economics Research Division, and Crops Research Division, Agrigultural Research Service, U. S. Department of Agriculture. Estimates for Alaska, California, Delaware, Hawaii, New Jersey, New York, Ohio, Oklahoma, and Washington not included.

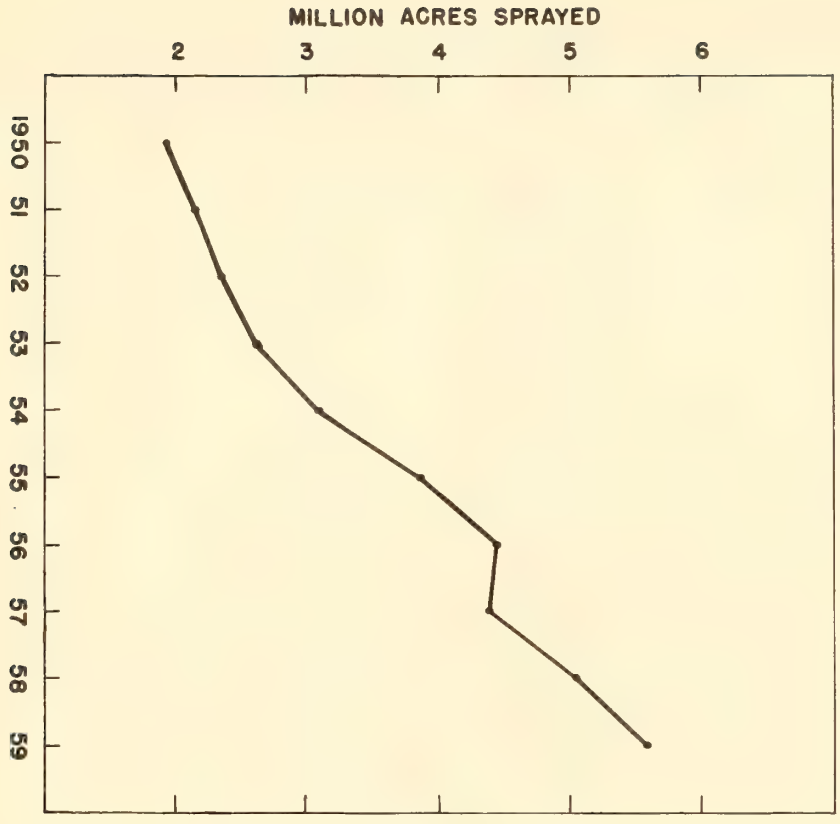
TABLE 3.--Estimated acreages treated for weed control in United States[1]

Year	Corn	Small grains	Grazing lands	All other	Total
	1,000 acres	*1,000 acres*	*1,000 acres*	*1,000 acres*	*1,000 acres*
1949	4,559	18,751	---	---	23,310
1952	8,150	16,792	2,192	2,629	29,763
1959[2]	15,720	19,770	5,828	[3] 4,114	45,432

[1] Estimates for 1949 and 1952 from U. S. Department of Agriculture, Statistical Bulletin 156, April 1955; 1959 estimates from unpublished survey data accumulated by the Federal Extension Service; Farm Economics Research Division and Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

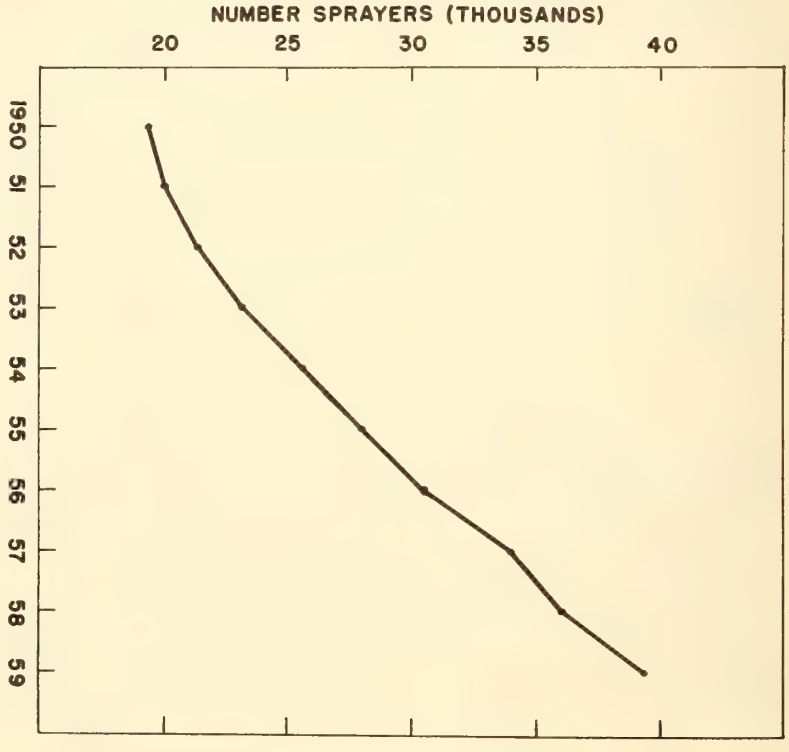
[2] Estimates for Alaska, California, Delaware, Hawaii, New Jersey, New York, Ohio, and Washington not included.

[3] Estimated 2.9 million acres noncrop land treated not included.



BN-10808-X

Figure 2.--Chemical Weed Control in grain crops in Minnesota, 1950-1959. (From Circ. 4, Section of Weed Control, State of Minnesota Department of Agriculture, December 1959.)



BN-10807-X

Figure 3.--Power sprayers used for weed control in Minnesota, 1950-1959. (From Circ. 4, Section of Weed Control, State of Minnesota Department of Agriculture, December 1959.)

TABLE 4.--Acreage corn treated for weed control in United States[1]

Year	Northeast	North Central	South	West	Total
	1,000 acres	*1,000 acres*	*1,000 acres*	*1,000 acres*	*1,000 acres*
1949	386	4,173	---	---	4,559
1952	844	7,276	865	165	8,150
1959[2]	566	13,384	1,666	104	15,720

[1] Estimates for 1949 and 1952 from U. S. Department of Agriculture, Statistical Bulletin 156, April 1955; 1959 estimates from unpublished survey data accumulated by the Federal Extension Service, Farm Economics and Crops Research Divisions, Agricultural Research Service, U. S. Department of Agriculture.

[2] Estimates for Alaska, California, Delaware, Hawaii, New Jersey, New York, Ohio, Oklahoma, and Washington not included.

TABLE 5.--Estimated cotton acres treated with defoliants and desiccants[1]

Year	Defoliants	Desiccants	Total
	1,000 acres	*1,000 acres*	*1,000 acres*
1940	trace	0	trace
1946	250	0	250
1952	2,391	120	2,511
1955	2,499	440	2,943
1958	3,103	1,595	4,698
1960	3,750	2,500	6,250

[1] Estimates kindly furnished by W. H. Tharp, Cotton and Cordage Fibers Research Branch, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

Significant progress was made during the past 5 years in the development of chemical methods for controlling soil-inhabiting plant parasitic nematodes on living plants, principally orchard and grove trees, but also in vineyards and nurseries. In studies in Arizona the treatment of the soil by introduction of 1,2-dibromo-3-chloropropane (DBCP) in the irrigation water has controlled the citrus nematode in heavily infested orchards and improved the economic returns as much as $90 per acre.

Another advance in the last 10 years was the wide adoption of methyl bromide as a treatment for the control of nematodes, soil insects, fungi, and weed seeds in seedbeds.

Other potential economic gains from the use of nematocides are illustrated by experimental results obtained in Georgia. The yields of sweet corn, cotton, squash, okra, beans, and cantaloupes were increased 15, 25, 85, 300, 125, and 70 percent, respectively, by nematocidal treatments.

Fungicides--Progress in the discovery and development of fungicides is shown by the following examples of fungicide usage to alleviate major disease problems.

Effective, low-cost protectant fungicides are available for seed treatment. Investment of a few cents per acre in these fungicides permits establishment of good stands of vigorous, disease-free seedlings of such plants as cotton, corn, small grains, peanuts, potatoes, sorghum, and vegetables (5). The investment of 30 cents per acre for fungicides to treat spinach seed has given increased yields valued at $50 to $300 per acre.

Most foliar and fruit diseases of orchard crops can be controlled satisfactorily by the use of currently available fungicides. Attractive fruit of high quality is available to the American housewife because of the availability and use of newer organic fungicides, both before and after harvest.

The devastating damages due to late blight of potatoes and tomatoes can be averted by skillful use of modern fungicides. Beginning about 1945, the use of a combination of ethylene bisdithio carbamates and DDT as an almost standard spray contributed markedly to doubling the yields of potatoes in the United States between 1939 and 1952 (1).

Fungicides are available to reduce markedly the damage caused by lima bean downy mildew, bunt, and dwarf bunt of wheat, onion smut, and blue mold of tobacco. A newer approach of plant disease control has evolved. This makes use of synthetic therapeutants in addition to antibiotics which permeate the host, then kill or suppress the pathogen, and thus prevent disease development even after infection has occurred. Organic and inorganic nickel compounds are under intensive study which show good promise as eradicant and protectant treatments for the cereal rusts.

The essentiality of crop-protecting chemicals in modern American farming cannot be questioned with any scientific justification. Enough specific data are not available to determine the total economic benefits that result from present uses of these chemicals, but undoubtedly marked reductions are being made in production costs wherever these materials are being used. In addition, improved quality and yields, lower labor cost, less risks of crop failure, and other benefits are being realized as improved pesticides are safely put to use.

PROBLEMS

Progress in the synthesis, evaluation, development, and use of pesticides is attributable to the research emphasis by public and private agencies. The research effort is far from adequate for unsolved and partly solved pest problems. To exploit adequately the potentials of synthetic organic chemicals for combatting plant disease organisms, weeds, and nematodes more efficiently, and without fear of harm to crop plants and to men and animals that eat them, there must be a marked increase in the number and support of scientists to do the necessary work.

In spite of the progress in the use of pesticides, farmers still endure an estimated loss of over 7 percent of crop productivity from plant diseases (5) and weeds cost the Nation's agriculture at least 10 to 15 percent of the productivity of all crop and grazing lands. In addition, weeds cost nonagricultural enterprises an estimated $1 billion annually. Data are incomplete for accurate estimates but nematodes are known to cause damages in excess of $300 million annually and in disease complexes with fungi they cause even greater losses. Sizeable losses in the quality of food and feed crops occur due to unregulated maturation and sprouting. The losses in yield due to premature drop of fruits or shattering of seed and frost damage indicate the real and potential losses that might be averted by the use of improved growth regulators.

There is still a critical need for improved economical chemicals to cope with many problems posed by various agricultural pests. This need can be fulfilled by expanding research to provide more knowledge so that we can design molecules for specific purposes and by utilizing more fully our present knowledge regarding the relations between molecular configuration and biological activity. New materials and techniques to control pests and modify plants must be continually sought by imaginative scientists so that a massive assault can be made on the problems along a broad front.

The following illustrate some of the critical unsolved problems which require more effective and cheaper chemicals and practices for their solution:

Weeds--No effective, economical weed control methods are available for annual broadleaved and grass weeds in soybeans, most vegetables, orchard crops, peanuts, and

a number of other crops. Many prevalent brush and herbaceous weeds of grazing lands, watersheds, and forests cannot be controlled with present herbicides and other methods. Johnsongrass, nutgrass, Canada thistle, quackgrass, field bindweed, tall larkspur, alligator weed, pond weeds, and other perennial weeds are not controlled satisfactorily. Effective, economical, and safe control methods are not available for controlling most aquatic and ditchbank weeds of farm drainage and irrigation systems.

These problems can be solved by strengthening research to synthesize and develop more efficient low-cost herbicides and then to devise methods for their practical and safe use. Basic understanding of the physiological, biochemical, and ecological requirements of the crop plants and weeds involved is prerequisite to devising chemical control methods for many weeds.

Growth Regulation--Many ornamentals cannot be propagated efficiently because of the lack of suitable root-inducing chemicals. Heavy losses of seed from many forage legumes could be prevented if satisfactory growth regulators were available to prevent shattering before harvest. Frost damage to fruit trees and quality deterioration of forage cut for hay could be prevented if satisfactory chemicals were developed for regulating maturation.

These needs could be fulfilled by additional research to understand the life processes involved in such phenomena as root induction, abscission of plant organs, and tissue maturation, and then synthesize and develop compounds to induce the plant response desired.

Nematodes--Control of nematodes on growing perennials with economical, low-phytotoxic nematocides is the biggest unsolved problem in nematology in spite of limited progress with current materials. Plant parasitic nematodes that limit efficient production of many agronomic and horticultural crops cannot be controlled because of high cost of present nematocides.

Increased research is needed to develop more effective, low-cost, and low-phytotoxic nematocides for controlling nematodes on living plants. Attention should also be given to the development of chemicals and methods for treating nematodes on roots several feet below the soil surface. Possibilities include low-phytotoxic chemicals which can be applied to the plant foliage, translocated to the roots, and then kill or repel nematodes. The synthesis and use of possible attractant chemicals in the soil might interrupt nematode feeding and reproduction activities sufficiently to make them disappear.

Plant diseases--No effective methods are available for controlling most root-rotting fungi. The general lack of plant resistance to the parenchyma-invading root-rotting fungi makes the need for chemical control methods especially urgent. Systemic seed-treatment chemicals and improved soil fungicides are needed for controlling damping-off and other seedling diseases of crop plants.

Bacterial plant diseases are not adequately controlled with present methods. The pear industry in most of the United States could regain its earlier stability and importance if effective, low-cost therapeutants were available to control fire blight. Present antibiotics appear erratic and too costly for the control of fire blight and certain other bacterial diseases.

Plant breeding for resistance to cereal rusts and other diseases needs to be supplemented with low-cost, translocatable, and effective therapeutants to kill the organisms within plants or inhibit their reproduction and spread. Concentrated effort should be made to complete toxicity studies on the promising nickel compounds as soon as possible.

Virus diseases are not controlled with present materials and methods. Research is needed to develop methods for screening chemicals capable of eradicating or arresting virus spread within plants.

The post-harvest development of bacterial diseases of fruits and vegetables, such as soft rot, cannot be controlled satisfactorily because of insufficient residue data on the antibiotics which are effective in controlling them.

THE JOB AHEAD

The increasing development and use of new herbicides, growth regulators, nematocides, fungicides, desiccants, defoliants, and other non-insecticidal compounds have created an urgent need for more research to gain an understanding of the actions of pesticides and their fate in and on crop plants. We should have enough knowledge about the behavior of pesticides in and on plants and soils to construct a balance sheet which accounts for a given chemical from application until its ultimate fate is known. The information is needed to permit the formulation of safe recommendations on the use of promising pesticidal chemicals which cannot be used at present because of the lack of adequate basic information on their actions, behavior, and disappearance from plants and soils.

A few pesticides that have been used for many years are now suspected of leaving possible residues on food commodities. Research is urgently needed to supply the necessary basic data and information for clearance or abandonment of these materials. Farmers may soon be denied several valuable and widely used chemicals unless research information is provided on residues and degradation products of the chemicals in the crops and food products concerned. Basic research on the actions and ultimate fate of chemicals is needed to fill an important gap in the present industrial and public agency research programs and also to reassure the public that the health of man and animals is being protected against harmful residues.

Expanded research is needed for:

- Developing improved materials and methods for combatting pests that will leave little or no chemical residues in or on raw agricultural commodities.

- Developing sensitive analytical procedures and methods for detecting quantitatively and identifying pesticide residues and degradation products in different tissues of various crop plants, in soils, and in irrigation water.

- Determining the effects of temperature, light, moisture, and other factors on chemical actions and on the disappearance of pesticides from plants and soils.

- Determining the entry, movement, persistence, and accumulation of pesticide residues in plants, including work on formulations and methods and times of application to minimize pesticide residues in and on crops.

- Determining the biochemical and physiological processes involved in the metabolism and degradation of pesticides.

- Determining the effects of chemicals on the chemical composition, taste, and other quality factors of food and feed crops.

Each pesticidal chemical must go through an obstacle course and measure up to certain standards before it can be used on agricultural crops. An admirable job has been done and continues to be done in cooperative industry-public agency research to provide adequate information to ensure the safe usage of agricultural chemicals from the standpoint of both public health and the crops involved. An additional hurdle which has been largely by-passed is more precise determination of the fate of pesticidal chemicals.

In the use of herbicides, therapeutants, and certain growth regulators there is a built-in safety feature in that excessive dosages cause serious injury to the crop plants involved. For this reason, considerable precision is necessary in applying the correct

dosage at the proper time and by a proved technique. In the development of chemical weed control practices the behavior and persistence of herbicides in soils must be understood to avoid possible injury to the crop plants being treated as well as succeeding crops in the rotation. One of the largest gaps in knowledge is possibly the effects of the chemicals on soil microorganisms, and the specific role that soil microorganisms play in the degradation of pesticides.

Future progress in the development and use of pesticides will depend upon cooperative basic research of scientists in public agencies and in the chemical industry. The continuation and expansion of basic research is the forward look and this holds the secret of successful development and use of crop-protecting chemicals in the future.

LITERATURE CITED

(1) Diamond, A. E., and Horsfall, J. G.
1955. Fifty years of fungicides. Ann. Appl. Biol. 42: 282-287.

(2) Dunegan, John C., and Doolittle, S. P.
1953. Plant diseases. U. S. Dept. Agr. Yearbook 1953: 115-120.

(3) Horsfall, James G.
1956. Principles of fungicidal action. Chronica Botanica Co. Waltham, Massachusetts. 279 pp.

(4) Large, E. C.
1940. Advance of the fungi. Henry Holt and Co., 488 pp., illus. New York.

(5) McNew, George L.
1959. Landmarks during a century of progress in use of chemicals to control plant diseases. Plant Pathology - Problems and Progress 1908-1959. The University of Wisconsin Press, Madison. 588 pp.

(6) Taylor, A. L.
1951. Chemical treatment of the soil for nematode control. Adv. in Agron. 3: 243-264.

(7) ———
1959. Progress in chemical control of nematodes. Plant Pathology - Problems and Progress 1908-1958. The University of Wisconsin Press, Madison. 588 pp.

(8) Taylor, A. L., and McBeth, C. W.
1941. A practical method of using methyl bromide as a nematocide in the field. Proc. Helminthol. Soc., Washington, D. C. 8: 26-28.

USE OF INSECTICIDES, ATTRACTANTS, AND REPELLENTS

E. F. Knipling[1]

Man's chief competitors for food are the insects. Insects and their close relatives are vectors of some of the most important diseases of man, animals, and plants. The soil harbors hundreds of insect species that feed on planted seeds or the roots of crops. Hundreds of other kinds suck the sap of plants, consume foliage, bore into the stems or damage the fruit. Still other insect pests attack food or fiber in storage. Every warm-blooded animal, including man, is subject to attack by one or more kinds of insects that are specific to the host while numerous others attack a wide range of animal hosts. Some insects live in water, others are adapted to live only in homes or other structures. Thus, among the 10,000 kinds of insects of economic importance in this country, we have control problems involving water, soil, cultivated crops, forests, rangelands, stored products, livestock, pets, homes, industrial establishments, and man himself. Everyone of the 175 million people of this Nation is faced with some insect problems and is, therefore, interested in methods of insect control.

Progress during the past two decades in dealing with insect pests of agricultural crops, livestock, and man, as well as vectors of human diseases, has been outstanding. The advances made are due to the close cooperation and combined efforts of scientists in various disciplines with Federal and State agencies and private industry. I know of no field of the biological sciences in which there has been closer cooperation between industry and public-supported research institutions.

Soil insecticides have come into use that control grubs, wireworms, termites, and other soil pests for periods up to 10 years or longer. Many materials are available for direct application to plants for the control of pests of fruits, vegetables, forage, grains, cotton, and other plants. Long-lasting residual insecticides and aerosols have been developed for use in homes to control many household pests and to prevent transmission of malaria and other diseases. A number of insecticides and repellents have been developed to protect livestock from insect attack. We have insecticides or insect repellents for direct application to the skin or clothing of man to prevent insect attack or the spread of dreaded diseases such as typhus and tick and mite fevers.

When we consider the scope of insect problems and the extensive use of chemicals employed for their control in such wide range of environments, it is apparent that potential hazards or conflicts of interests are to be expected. However, before commenting further on this major and continuing problem which confronts entomologists, I would like to state that the over-all picture of hazard due to insecticides painted in popular or even in some scientific publications by some alarmists is more imaginary than real.

Considering the many insect control chemicals involved and their widescale use, I regard the record of safety in insecticide use to be outstanding. Insecticides are responsible for fewer accidental deaths than are many of the common household chemicals or over-the-counter drugs. There is not a single proved instance of chronic poisoning in man due to insecticide residues in foods (Hayes (3)). Local adverse effects to fish and wildlife may result from the use of insecticides and every effort must be made to avoid such losses. But contrary to implications by some writers, I have seen no evidence of a significant decline in the Nation's resources of fish and game that can be attributed to insecticides even in agricultural areas where there is extensive use of insecticides. Many instances of adverse effects to beneficial insect parasites and predators resulting from the use of insecticides have occurred. Fortunately, however, as reported by Lincoln (9), this effect appears to be temporary and there is no evidence of a lasting upset in balance between beneficial and destructive insects even in cotton fields where insecticide usage is most extensive.

[1]Director, Entomology Research Division, Agricultural Research Service, U.S.D.A.

It is not my purpose to minimize the real and potential significance of insecticide hazards. The importance of producing high-quality foods free of hazardous residues cannot be over-emphasized. However, I do wish to urge sane and objective consideration of all factors relating to the use of insecticides. It is necessary that this be done if this Nation is to take full advantage of scientific advances that have been made in the development and use of insecticides and those that can be expected in the future. Growing public apprehension over pesticide use is based in large measure on inaccurate and misleading reporting by uninformed writers. This leads to stringent laws and rigid interpretations of laws regulating the use of insecticides or other pesticides. As a result, we are facing a growing critical problem in our efforts to maintain satisfactory insect control methods and to develop new ones. Under existing law, use of a particular insecticide must not only be nonhazardous but must also meet other requirements of law.

It should be emphasized that hazards associated with the use of insecticides is not a new problem to entomologists and associated scientists. They have always been conscious of the need to reconsider this important aspect of research on the control of insects with chemicals. The hazard problems created by arsenical insecticides to plants and soils, to livestock in dips or as feed contaminants, to bees in orchards, to parasites of destructive insects, and to man directly as residues on foods or as poisons in homes, emphasized the necessity to consider potential hazards in connection with research and subsequent recommendations for the use of the newer chemicals.

However, no one could have predicted the many complex problems that would arise in connection with the use of the newer type insecticides. When the newer chemicals came into extensive use 15 years ago, we could not even approximate a fair estimate of the time, effort, and cost that would be involved in a full investigation of all aspects that are now regarded necessary or desirable before a new chemical can be recommended for widescale use in insect control. The growing complexity of this problem soon became apparent, however, and the need to give it due attention was urged repeatedly by entomologists and insecticide chemists. The public in general and many public officials just did not grasp the significance of the insecticide residue problem or the important role that insecticides would play in modern agriculture and public welfare.

The public is entitled to both high standards of insect control and high standards of safety in the use of insect control procedures. If the public is convinced that the many highly effective and proved methods of insect control do not provide the standards of safety desired, it must be prepared to support research to develop more acceptable methods or to accept short supplies, inferior quality, and higher prices for many foods.

With the exception of insect problems of forests and stored products, the Entomology Research Division has been assigned primary responsibility for the Department's research program on insects. The available resources in terms of number of scientists and supporting personnel in this Division are lower today than they were in 1945, the beginning of the era of new insect control chemicals in agriculture. Let's analyze this situation in relation to the following developments all of which have placed new demands on the Division. During these 15 years, we have been faced with (1) public interest in and requests for information on the many new chemicals developed by industry, (2) an unprecedented expansion of the use of insect control chemicals by farmers, (3) the appearance or spread of several major introduced pests, (4) new farm practices and great diversification of agriculture in new areas which influence pest abundance, (5) the passage of stringent laws, such as the Miller Amendment, which necessitated reevaluation of every insecticide recommendation, including many that had been followed by growers for 25 to 50 years, and which prescribed requirements that greatly increased the research effort needed to develop new recommendations, (6) a general expansion of regulatory programs designed to control or eliminate insect pests, (7) a general expansion of agricultural research in most other areas except entomology, (8) public demands for new and safer approaches to insect control, such as by biological means, and (9) the appearance of a hundred or more kinds of insects that developed strains resistant to the newer insecticides for which substitute chemicals were necessary.

I wish to take this opportunity to commend the scientists of the Division for their efforts to carry out the Department's responsibilities for the entomology phases of farm research in the face of such demands and handicaps. In so doing, I also wish to acknowledge the assistance and cooperation of many individuals in the Department, State Experiment Stations, and industry.

SPECIFIC EXAMPLES OF PROGRESS AND PROBLEMS

In considering progress and problems relating to the use of insect control chemicals in crop and livestock production, only a few examples can be cited here.

Until the newer insecticides came into being, the toll taken by insects on a number of crops was not fully appreciated. For example, when DDT became available to potato growers for controlling the green peach aphid, wireworms, and other pests, the yield of potatoes virtually doubled in many areas. Increases in yield of marketable potatoes during recent years are due to improvements in a number of agronomic practices. However, without effective insect control chemicals and their proper use by the grower near maximum yields and high quality cannot be achieved.

FORAGE AND GRAIN INSECTS

The European corn borer is the major pest of corn in the main Corn Belt. Losses due to this introduced pest average about $100 million annually. DDT treatment of susceptible varieties for protection against 1st generation borers may result in over 25 percent increase in yield.

The high cost of control for the corn borer and the residues associated with the use of insecticides are important problems in dealing with this insect. When DDT, the most economical insecticide, is used for corn borer control, the farmer cannot feed the stover to dairy cows or beef animals to be marketed for slaughter.

Alfalfa is our most important cultivated forage crop. It is subject to attack by several important insect pests, including the alfalfa weevil, spotted alfalfa aphid, pea aphid, potato aphid, plant bugs, and spittle bugs. Through the use of insecticides now available, it is not uncommon to increase yields of alfalfa by 50 percent as well as to improve the quality of the hay produced. However, the growers are faced with the problem of controlling the insects effectively and economically without leading to traces of residue in milk of dairy cows consuming the forage. The recent reduction of the tolerance of heptachlor and its epoxide to zero was a serious blow to alfalfa growers particularly where the alfalfa weevil and spittle bugs are major pests.

An excellent example of the possibilities of integrating chemical and biological control procedures in dealing with an insect problem can be cited for the spotted alfalfa aphid and the insecticide Systox. R. F. Smith, V. M. Stern, R. van den Bosch, and K. S. Hagen of California have given special attention to this approach to the control of this introduced insect, and published a series of three papers (11, 12, 13) describing their success in achieving integrated biological and chemical control. Through proper timing in the application and with the proper dosage of this sytemic insecticide, it is possible to reduce aphid populations without serious adverse effects on parasites and predators which continue to exert their influence in preventing population buildup.

FRUIT INSECTS

Experience has shown that insecticides are essential for the commercial production of most fruit crops. For many years, commercial growers of apples relied on arsenicals for controlling the codling moth. DDT proved to be a boon to apple producers and it

seemed that DDT was the long-awaited answer the fruit grower wanted. However, several important side problems emerged which are typical of similar developments resulting from the use of insecticides on other crops. The regular use of DDT in orchards led to an increase in mites and certain other pests because of the destruction of their natural enemies. DDT-resistant strains of the codling moth appeared after 8 to 10 years of use. Although DDT residues in relation to apples as a human food posed no serious problem, they have created a special problem in relation to the use of apple pomace as a livestock feed. DDT levels, in the range of 5 to 7 p.p.m. on fresh apples, will lead to levels in the pomace up to 25 p.p.m. or higher after extraction of apple juices. Pomace has been used to some extent as a feed for dairy and beef animals but such use must be discontinued. Thus, apple pomace has become a liability rather than an asset in the processing of apples. This is just one example of the many ramifications of the residue problems that could be cited. Substitute insecticides for codling moth control have been developed through the efforts of State, Federal, and industry workers that can obviate this residue problem. However, substitute insecticides may have both advantages and disadvantages. They may avoid residues in the pomace but may be more harmful to certain beneficial insects, to fish and wildlife, or be more costly. It is not a simple matter to find and develop an effective substitute insecticide that meets all of the standards desired.

A few outstanding advances have been made in the use of attractants for fruit insect control. As an outgrowth of basic studies on the nutritional requirements of fruitflies conducted cooperatively by the California Agricultural Experiment Station and the Hawaii Fruit Fly Laboratory of the Entomology Research Division, a protein hydrolysate bait spray was developed for eradication of the Mediterranean fruitfly in Florida in 1956 and 1957. The Hawaii laboratory has also cooperated with the Division's Pesticide Chemicals Research Branch in the development of a number of highly active male lures for the Mediterranean, melon, and oriental fruitflies. A highly effective oriental fruitfly lure, methyl eugenol, has been studied extensively by the Hawaii laboratory and plans are now under way to attempt eradication of this insect from one of the Pacific islands by destroying all of the males attracted to this lure.

Aside from the possible use of lures for direct control of fruitflies, they are essential as a means for detecting low level infestations of fruitflies, as was demonstrated in the successful Mediterranean fruitfly eradication program in Florida.

COTTON INSECTS

Much has been said about the importance of insects in the production of cotton. The benefits derived from the use of insecticides to control cotton insects are well known to the cotton grower.

For the last 20 to 37 years yield records have been obtained at Florence, S. C., Tallulah, La., and Waco, Tex., in untreated experimental plots and similar plots treated with insecticides. Since the new insecticides came into use which control a wide variety of cotton pests as well as the boll weevil, the yields in treated plots have averaged about 50 percent increase over check plots.

The boll weevil is the most important pest in the main Cotton Belt, but it does not occur or is not a factor in cotton production in the Western States. However, experimental plots in Arizona consistently show substantial increases in yield through the use of insecticides for the control of plant bugs, cabbage loopers, salt marsh caterpillars, thrips, and other cotton pests. The Arizona Agricultural Experiment Station has shown an interesting correlation between the steady increase in yields of cotton per acre during recent years and the amount of insecticide applied per acre.

In the production of cotton and a number of other crops, many advances have been made during recent years in soil and water management, use of fertilizers, high-yielding varieties, and disease control. Cotton insect control alone will not assure high yields of cotton. However, maximum potential in cotton production efficiency cannot be realized without good insect control. In fact, practices which are essential for high yields may greatly intensify cotton insect problems unless adequate control measures are applied.

INSECTS AFFECTING MAN

Developments during and subsequent to the war years in the control of vectors of human diseases have been outstanding. The residual spray approach to malaria control, first investigated for the military by the Orlando, Fla., laboratory of the Entomology Research Division, has been fully developed and utilized by many agencies. This method now forms the basis for a coordinated world-wide malaria eradication program sponsored by the World Health Organization, International Cooperation Administration, Pan American Sanitary Bureau, and local health agencies in many parts of the world. Typhus, one of the most devastating diseases of man, especially during periods of strife, is currently no longer a threat to mankind because of the basic developments by the Orlando laboratory on louse control insecticides. These and many other similar developments in insecticides, attractants, and repellents have been of inestimable value in raising standards of health and welfare of man throughout the world.

However, the extensive use of chemicals for insects affecting man has led to many problems during the past two decades. Hazards are of special significance when we are dealing with chemicals for direct application to man or for use in his home. Here we are providing maximum opportunity for acute or chronic toxic effects through exposure to an insecticide. Careful study and appraisal of the hazard of a chemical is absolutely necessary before a material can be employed. At the same time, however, such appraisal must be objective with due regard for the need.

It is perhaps fortunate that DDT was under development during the war. Responsible officials concerned with vector-borne diseases were placed in a position where it was necessary to weigh the known hazard of the disease against the potential hazard of the chemical. I venture to express an opinion that if the same conservatism in appraising potential hazards and the same legal restrictions in the use of insecticides had existed during World War II which now prevail, DDT would not have been approved for direct application to man or for application as residual sprays in homes. Yet, this insecticide has been applied as a 10-percent dust inside of the clothing of hundreds of millions of men, women, and children by military and public health officials and has been applied as residual sprays in as many homes, without one known case of toxic effects to individuals exposed to such intimate insect control practices. As a result, literally millions of lives have been spared and hundreds of millions of people have escaped diseases.

In the light of the extensive safe use experience with DDT, backed by the classical experiments by Hayes (4), it would seem reasonable to regard DDT as one of the safest insecticides ever developed. However, this chemical has been under constant fire by critics of the use of insecticides.

The problem of insect resistance to insecticides has had a major impact in the field of medical entomology. Widespread resistance to DDT and other insecticides has occurred among houseflies, mosquitoes, body lice, bedbugs, and a number of other insects. More recently, resistance to organic phosphorus compounds has appeared among several insects affecting man. The impact of the resistance problem among so many medically important insects against all types of insecticides should be carefully noted in our appraisal of the potential significance of the insecticide resistance problem among agricultural pests in the years ahead.

LIVESTOCK INSECTS

Many of the same types of insects affecting man also attack livestock. In adapting the insecticides which proved so useful on man for the control of livestock pests, we encountered the first serious residue problem involving milk. Howell, et al. (5) of Oklahoma demonstrated that the application of DDT to dairy cows for insect control resulted in several parts per million of the insecticide in milk. Subsequent research by a number of investigators confirmed these findings. Moreover, the phenomenon of insecticide storage in meat and milk has been found to occur with most of the insecticides when the compounds are applied externally or ingested by the animal.

During the past 12 years the Entomology Research Division has placed major emphasis on the insecticide residue problems in relation to meat and milk. Much of this research has been carried forward by the Kerrville, Tex., livestock insect laboratory in cooperation with the Animal Disease and Parasite Research Division. Many formal cooperative programs have been jointly financed by industrial companies and the Department for the purpose of studying the relationship of contemplated insecticide use for livestock and forage pests and the appearance of insecticide residues in meat and milk. This subject will be treated more fully by Westlake and San Antonio and Radeleff and Bushland in the papers prepared for this symposium.

As a result of such investigations, clearance has been obtained for a number of insecticides for application to meat animals and for some to be applied to dairy animals. However, the research has also yielded residue data which led to the discontinuance of recommendations for several compounds important in dairy production. Today the insecticides and repellents we have for protection of dairy cows from most insects and ticks are no better than they were 25 years ago.

The use of systemic insecticides for the control of cattle grubs has been pioneered by the Kerrville, Tex., and Corvallis, Oreg., laboratories of the Entomology Research Division. Through the cooperation of these laboratories, industrial research groups, many State agricultural experiment stations, and the Animal Disease and Parasite Research Division two animal systemics, ronnel and Co-Ral, are now in practical use for cattle grub control and other more promising materials are under investigation.

BASIC DEVELOPMENTS IN CHEMICAL RESEARCH

I have said little about the more basic achievements in the chemical aspects of insecticide development and use. It is not possible in this general treatment of the subject to properly credit the many chemists in industry and in Federal and State institutions who have discovered and developed the many and varied insecticides, attractants, and repellents in use today. Most of the materials in use have come from industrial laboratories, but public-supported institutions, including the Citrus Experimental Station, Riverside, Calif., the University of Illinois, and the Pesticide Chemicals Research Branch in the ARS Entomology Research Division, have made valuable contributions in the development of new materials. Industry has borne most of the cost of early research to establish the biological effectiveness of new compounds and to obtain basic information on their toxicity to animals. However, the cooperation of entomologists and chemists in State and Federal laboratories throughout the country has made it possible to obtain necessary information on the biological effectiveness of compounds for specific insect pests and to obtain residue data needed to establish tolerances.

In spite of the need for additional information on the nature and fate of insecticides in raw agricultural products, the progress made through this cooperative effort by many individuals has been outstanding and has led to the establishment of over 2,000 tolerances involving a wide variety of insecticides, and commodities.

The Pesticide Chemicals Research Branch of the ARS Entomology Research Division has borne the brunt of the Department's responsibility in meeting the insecticide residue problem. In addition to this phase of the Branch program, however, some basic studies on the relationship of chemical structure and biological activity have been undertaken which have led to several important contributions. Much of the development in the field of synergists for pyrethrum stems from early research on the structure of the synergistic components in sesame oil (Haller, et al., (2)). The elucidation of the chemical structure of the pyrethrins (LaForge and Haller (6), LaForge and Barthel (7), and LaForge and Soloway (8)) and subsequent synthesis of allethrin and other pyrethrum-type compounds (Schechter, et al. (10)) represent important achievements. The best insect repellent available today, diethyl toluamide was discovered by chemists in this Branch. The research on synthetic attractants for Mediterranean and melon fruitflies already referred to has been highly productive.

The aerosol principle of applying insecticides, developed by Goodhue and Sullivan (1), represents one of the outstanding achievements in the field of insect control. It has not only led to important developments in methods of insect control, but an entirely new industry has emerged having a retail value estimated at $500 million annually.

ENTOMOLOGY RESEARCH NEEDS

The Entomology Research Division has made a determined effort to orient its programs to help meet the increasing number of pressing problems. However, with a steady decline in research resources it has been impossible to meet current problems and at the same time begin laying a firm foundation for better long-range solutions to insect control.

The many ramifications of the residue and other hazard problems began emerging as long as 15 years ago. Since then no less than $500,000 of the annual research budget has been shifted in efforts to obtain necessary residue and related information. In view of the hazard and insecticide resistance problems, it became apparent that a reappraisal of the usual approaches to insect control was needed. As a result, every effort was made to undertake more basic investigations on chemicals and to explore new chemical approaches such as attractants as well as to give more attention to nonchemical methods of control with particular emphasis on insect pathogens. Two pioneering research laboratories, one on insect physiology and the other on insect pathology, were established at Beltsville and additional research in these areas were undertaken in field laboratories. Substantial shifts in the overall research program were also made to explore the possibilities of utilizing the sterile male method of insect control.

These efforts cited above have yielded encouraging information. However, they are totally inadequate in relation to the needs if rapid progress is to be made.

In my opinion the opportunities for developing new and safer methods to control insects are virtually unlimited. The extent to which improvements can be made will depend on the research support provided to permit adequate exploration of many basic and applied aspects that obviously need attention. With such support and with full cooperation of entomologists, chemists, animal toxicologists, pharmacologists, wildlife biologists, engineers, plant breeders, and scientists in other disciplines, both in public-supported and private institutions, there is every reason to believe that substantial and rapid progress can be made. However, to obtain maximum benefits from any research effort, objective appraisal of the findings are essential and relative values involved must be carefully weighed in applying the control measures in some instances.

It is not my intention to discuss in detail here the type of research on insects that is most likely to achieve the objectives we seek. However, I would like to list a few areas of research that on the basis of current information and judgment would seem to be highly productive.

1. New types of chemicals should be developed that are biologically active against destructive insects but inactive to or will have minimal adverse effects on other forms of life. Both basic and applied research leading to the development of such chemicals are needed. The possibilities of finding chemicals which are specific in action against insect hormones or which produce antimetabolic effects that will prevent normal insect development or behavior should be considered.

2. Research on insect attractants whether they be derived from insect foods, from insects themselves, such as sex attractants, or from other sources would seem to offer unusual opportunities for developing natural or synthetic attractants highly active against specific insects without affecting honey bees, insect parasites, and predators, or other beneficial organisms.

3. A thorough exploration of diseases that affect insects should be undertaken. Any organisms such as bacteria, viruses, fungi, and protozoa, that are highly virulent, should

be considered for possible practical application. Insect pathology is a relatively young science pioneered in this country by the University of California, the United States Department of Agriculture, and other institutions but there is every reason to believe that this approach to insect control might achieve the desired action against a number of insects without hazard to plants, soils, beneficial insects, or to higher animals, including man.

4. Maximum utilization of parasites and predators of destructive insects through explorations and subsequent introductions to obtain establishment or through mass production and releases in sufficient numbers and frequency to exert direct control of destructive pests, needs further research attention. More attention should also be given to the preservation of the many beneficial insects now in the crop environment by developing types of insecticides and methods of use that will not seriously affect their ability to help keep destructive pests under reasonable control.

5. A broad and long range program with the full cooperation and assistance of plant breeders should be undertaken promptly for the purpose of searching for insect resistant germplasm and subsequent development of crop varieties which are resistant to the major insect pests. There is reason to believe that a high level of resistance to insects can be found and incorporated into many crop varieties. Where successful, this method of insect control can obviate all hazards at virtually no cost to the grower.

6. The male sterility approach to insect control which proved so successful in eliminating the screwworm from Curacao and from the Southeastern United States needs investigation for many other major insect pests. This principle of control is basically sound and the extent to which it can be applied to help control or regulate insect and other noxious animal populations will largely depend on the skill and ingenuity of the investigators. Sexually sterile but otherwise sexually vigorous and fully competitive insects in a natural population will exert greater effect in reducing the reproductive potential of such population than can be achieved by destroying a like number of individuals. Thus, it might prove highly productive to concentrate substantial research effort on practical ways to achieve the desired type of sexual sterility instead of finding ways to kill the insects directly. Other ways of utilizing insects for self-destruction also need to be explored. The development of genetically inferior strains or the use of insects as vehicles for the spread of insect pathogens and other agents of destruction may also provide new ways to control specific pests.

In the planning of research to solve insect problems in the future as with many other fields of science, it is necessary, regardless of the approach, that there be developed and maintained a proper balance between basic and applied research. In addition, it is necessary to carry forward a research program sufficiently broad in scope so that all aspects having an important bearing on the final solution to the problem can be investigated concurrently. Such balance has not existed in the past even in the field of chemicals. We have made outstanding advances in developing highly effective insecticides for insect destruction which has returned great dividends to growers and to the public in general. However, we are not in a position to fully consolidate the gains that have been made because of the lack of information on such aspects as the amount and significance of residues in foods, the effect of the chemicals on other organisms in the environment, how to apply the material with maximum precision and in minimum amounts, how to integrate their use to supplement other methods of control, as well as many other aspects that determine maximum utility and safety in the use of chemicals.

LITERATURE CITED

1. Goodhue, L. D. and W. N. Sullivan. Method of applying parasiticides. U. S. Patent 2,321,023. June 8, 1943; appl. July 29, 1941. Assigned to Secretary of Agriculture.

2. Haller, H. L., F. B. LaForge, and W. N. Sullivan. Some compounds related to sesamin: their structures and their synergistic effect with pyrethrum insecticides. Jour. Org. Chem. 7:185-188. 1942.

3. Hayes, Wayland J., Jr. Hazards in the use of insecticides. Proceedings 39th Mid-Year Meeting Chem. Spec. Mfg. Assn. May 1953.

4. _____ The effect of known repeated oral doses of chlorophenothane (DDT) in man. Jour. Amer. Med. Assn. 162(9): 980-897. 1956.

5. Howell, D. E., H. W. Cave, V. G. Heller, and W. G. Gross. The amount of DDT found in milk of cows following spraying. Jour. Dairy Sci. 30, 717-721. (1947)

6. LaForge, F. B., and H. L. Haller. Constituents of pyrethrum flowers. VI. The structure of pyrethrolone. Jour. Amer. Chem. Soc. 58: 1777-1780. 1936.

7. _____ and W. F. Barthel. Constituents of pyrethrum flowers. XVIII. The structure and isomerism of pyrethrolone and cinerolone. Jour. Org. Chem. 10:114-120. 1945.

8. _____ and S. B. Soloway. Constituents of pyrethrum flowers. XXI. Revision of the structure of dihydrocinerolone. Jour. Amer. Chem. Soc. 69: 2932-2935. 1947.

9. Lincoln, Charles. Pesticides and wildlife symposium. 26th Ann. Mtg. Nat'l. Agric. Chem. Assn. 1959.

10. Schechter, Milton S., Nathan Green, and F. B. LaForge. The synthesis of cyclopentenolones of the type of cinerolone. Jour. Amer. Chem. Soc. 71:1517. 1949.

11. Smith, R. F. and K. S. Hagan. The integration of chemical and biological control of the spotted alfalfa aphid. Impact of commercial insecticide treatments. Hilgardia 29: pp. 131-154. 1959.

12. Stern, V. M., R. F. Smith, R. van den Bosch, and K. S. Hagan. The integration of chemical and biological control of the spotted alfalfa aphid. The integrated control concept. Hilgardia 29: pp. 81-101. 1959.

13. _____ and R. van den Bosch. The integration of chemical and biological control of the spotted alfalfa aphid. Field experiments on the effects of insecticides. Hilgardia 29: pp. 103-130. 1959.

USE OF ANTIBIOTICS, HORMONES, TRANQUILIZERS, AND OTHER CHEMICALS IN ANIMAL PRODUCTION

N. R. Ellis, R. E. Davis, and C. A. Denton[1]

The use of feed adjuncts or additives in the current sense of the term is a development of the past 20 years. The preceding 20 years witnessed the development of information on vitamins and on a number of mineral elements. Antibiotics, organic arsenicals, tranquilizers, and surfactants were unknown insofar as livestock feeding was concerned. Hormones were just on the horizon.

The interest in hormones 20 years ago was largely from the standpoint of physiological function. Only a few investigators were studying the effects of hormone preparations in livestock. Thyroid regulation appeared to be an attractive field. Among the studies in this area, that of Andrews and Bullard (1) appears significant to this review. In 1940, they reported results of some studies on the effects of partial thyroidectomy on the fattening of steers. This represented an effort to alter the normal hormonal pattern in finishing animals for market. By circumstances or otherwise it paved the way for developments in direct feeding of thyroidal compounds rather than surgical treatment.

The World War II years witnessed initial research on feeding compounds to various classes of livestock having thyrogenic properties. Control of thyroid status seemed to offer great possibilities in animal production through the use of products that produced hyperthyroidism on the one hand and hypothyroidism on the other.

During the 1945-1950 period numerous research studies were reported on the use of synthetic goitrogens that produced varying degrees of hypothyroidism. The more common compounds were the several forms of thiourea and thiouracil. Concurrently and extending up to 1955, there was much interest in hyperthyroid-producing materials--principally thyroxine. Much work went into the development of a product variously known as iodinated casein, thyroprotein, and other names carried thyroxine activity. Synthetic thyroxine also made its appearance. Much of the interest was in the feeding of dairy cows. Recently, there has been reactivation of research in swine.

During the 1943-1945 period a number of research reports appeared on the use of estrogens in poultry fattening, followed by an apparent lull lasting until about 1950, even though a new drug application for the use of stilbestrol implants became effective in 1947.

Extensive research with stilbestrol and other sex hormones began about 1950 on cattle, sheep, and swine, and was resumed on poultry in 1951. Since that date there has been widespread activity in this area. In 1954, the oral administration of stilbestrol in beef cattle came into general use through the granting of a new drug application. The permit for marketing of stilbestrol pellets for implantations in the ears of beef cattle became effective in 1955. In the case of sheep, application was allowed for oral administration in 1957, and for pellet implantation in the ear in 1959. Various groups of hormone products that have been studied are listed below:

Some Hormones Tested in Feeding Experiments

Thyroidal	Female sex
Thyroxine	Estradiol
Iodinated protein	Dienestrol
Triiodothyronine	Diethylstilbestrol
	Hexestrol
Goitrogens	Progesterone
Thiouracil (various forms)	
Thiourea (various forms)	Male sex
1-methyl, 2-mercaptoimidazole	Testosterone
	Methyl androstenediol

[1] Associate Director, Animal Husbandry Research Division, Nutritionist and Leader, Beef Cattle Nutrition Investigations, and Chemist (Biochemist) and Leader, Poultry Nutrition Investigations, respectively, Animal Husbandry Research Division, Agricultural Research Service, U. S. Department of Agriculture.

The subject of hormonal relationships and applications was reviewed in Publication 266 of the National Research Council in 1953 (10) and again in Publication 714 in 1959 (11).

The developments in the use of antibiotics largely date from 1949 when fermentation residues were found to have growth-promoting effects in poultry and swine apart from the vitamin B_{12} content. The disease level concept was introduced to explain the effectiveness of antibiotics in correcting slow growth and generally in accelerating growth rates. A great number of researches followed the early observation and in a remarkably short period the use of antibiotics was adopted for general commercial feeding practices. Numerous reviews on antibiotics in nutrition have been published. The list of antibiotics is an ever-expanding one. Some 17 antibiotics that have been noted in research papers are given below:

Some Antibiotics Tested as Feed Additives

Actidione	Neomycin
Bacitracin	Oleandomycin
Chloramphenicol	Oxytetracycline
Chlortetracycline	Penicillin
Erythromycin	Polymyxin
Fumagillin	Streptomycin
Gramicidin	Subtilin
Hygromycin	Tyrothricin
Ilotycin	

Of relatively recent interest are tranquilizers, with greatest emphasis on beef cattle and sheep. Some of the tranquilizers that have been tested in feedlot studies are shown below. There are many more of these that have been prepared and tested pharmacologically.

Some Tranquilizers Tested as Feed Additives

Chlorpromazine	Rauwolfia
Ethyl isobutrazine	Reserpine
Hydroxyzine	Tetrahydrozoline
Mepazine	Trifluomeprazine
Perphenazine	Trifluoperazine
Prochlorperazine	

Various other classes of feed additives, the principal group being the arsenicals with two types--the arsonic and the arsanilic acids--are given below. The beneficial effects of feeding arsenicals to chickens were observed as early as 1877 and were rediscovered in 1946.

Other Feed Additives Tested in Feeding Experiments

Organic arsenicals	Enzymes
Arsonic acids	Surfactants
Antioxidants	Live rumen cultures

Fowler's solution was once a widely used tonic for livestock. Research in recent years has largely revolved around organic arsenicals in the feeding of growing poultry and swine. The main concentration of research dates from about 1951 and the major interest has been in poultry.

Other classes of materials include surfactants, enzyme preparations, live rumen cultures, and others. Research interest has not been as widespread although numerous reports have appeared in the last few years. While antioxidants do not function in the way

most of the other compounds do, they have a place as feed additives for protection of vitamins and fats against oxidation. Surfactants have taken a somewhat limited place of interest as have enzymes. Both would appear to function as digestive aids in promoting greater efficiency in feed utilization.

A great number of compounds within the general groups indicated have been developed synthetically or biologically in recent years. In the earlier developments the concern was primarily for possible use in human medicine. The great interest in livestock feed additives has spurred biochemists to develop compounds primarily for this purpose.

Thus far we have attempted to portray the chronological development research-wise of the feed additives problem over the last 20 years. In several cases the quickness with which research findings have been applied to feeding practice has been phenomenal, although in others there has been little or no application. The reasons for adoption of some of these research findings to production practices undoubtedly rest primarily on the financial returns. These increased returns are to be found in the acceleration in rate of gain, in feed savings, and sometimes in decreases in death losses. We will undertake to point up some of these factors in use of feed additives.

At the onset, it must be pointed out that, in summarizing the material, there is the hazard of over-simplification, especially in the presentation of average figures and in drawing conclusions. It must be kept in mind that widely divergent figures are contained in the tables of average results. Furthermore, they have not been evaluated for statistical significance.

BEEF CATTLE

The use of diethylstilbestrol in the fattening of beef cattle was adapted to feedlot practice remarkably quickly and extensively. Following acceptance of the new drug application in 1954, the oral feeding procedure was soon used on a widespread scale. Within a 2-year period estimates indicated that about 60 percent of the cattle in feedlots were receiving the compound. Subsequently, the figure rose to 70 percent and more recently to 80 percent. The distribution and rationing procedure provides 10 mg. per animal per day. Many feeding trials have been conducted in which rates of gain, feed consumption, and carcass data have been obtained. The gain stimulation and feed savings have been consistently favorable as a general rule. Carcass merit has been maintained in most comparisons. The advantage to the producers has rested in the shorter feeding period and reduced feed requirement. A summary by Burroughs (4) from Iowa State University (Table 1) shows an average increase in rate of gain of 13 percent and a feed saving of 10 percent for fattening cattle receiving 10 mg. of stilbestrol as compared to control groups. His figure for increased returns per animal is $6.94. These favorable results undoubtedly explain the widespread practice that use of the drug has attained.

Implanting pellets in the ears of feedlot cattle have likewise increased rate of gain and decreased feed requirement. Comparisons of the effectiveness of different amounts of stilbestrol indicate that implantation of 36 mg. gave a gain advantage of 15 percent and a feed saving advantage of 10 percent. Carcass grades were slightly lower, however. Whether the financial return is as favorable for pellet-treated animals as for orally treated is not clear.

Numerous experiments have been conducted on other hormone products. Hexestrol, administered orally, has given results about equal to those just cited for stilbestrol to which it is closely related chemically. Oral feeding of dienestrol has been effective, also. Implants of progesterone, combined with estradiol, have given responses approaching those of stilbestrol. Besides the feedlot studies, there has been much interest in hormone treatment of young cattle on pasture as well as wintering studies. Positive responses have been obtained especially with stilbestrol.

TABLE 1.--Summary of Experimental Comparisons of Feed Additives in Beef Cattle Rations

(From Iowa State University - AH 805)

Feed additive	Expt'l comparison	Gain increase	Feed saving	Return per animal
	Number	*Percent*	*Percent*	*Dollars*
Stilbestrol - oral				
Fattening ration	105	13	10	6.94
Growing ration	58	12	10	5.63
Stilbestrol - implant				
24 mg.	35	9	6	.19*
36 mg.	45	15	10	3.00*
Hexestrol - oral	14	13	10	
Dienestrol - oral	4	9	8	
Progesterone - estradiol implant	13	12	8	2.40
Tranquilizers	72	3	1	.90
Enzyme (Agrozyme)	12	7	4	3.05
Antibiotics	112	4	3	3.16

*Incomplete comparison.

The summary from Iowa State University shows that antibiotics, mainly chlortetracycline and oxytetracycline, promoted some increase in gain and in feed saving averaging 4 and 3 percent, respectively, in fattening beef cattle. Actually, research reports have been somewhat conflicting as to effects of the antibiotics on the rumen flora and on the digestive process. The level and type of antibiotic used and the proportions of roughage and concentrates making up the ration all appear to be factors. It appears that relatively low levels of an antibiotic that does not unduly upset the rumen flora balance and suitable rationing does promote some increase in rate of gain and in feed economy. There has been some benefit noted in use of antibiotics in supplemental feeding of suckling calves.

The use of tranquilizers first came into prominence in connection with shipment of feeder calves and their adjustment to the feedlot. Subsequently, tests have been made on responses during the feeding period. The data summarized in Table 1 do not suggest any great advantage in the use of tranquilizers thus far tested.

Several enzyme preparations that have been tested have given relatively modest positive responses. Undoubtedly, before full appraisal is made, additional studies on more preparations will be needed.

With beef cattle and other species as well, many tests on various combinations of feed additives, such as hormones combined with antibiotics, have been made. The number of such combinations is too large to attempt any detailed analysis at this time. Frequently there are added benefits.

The effect of the various feed additives on quality and grade of beef carcasses has been much debated. It appears that some lowering of carcass grades frequently results from the use of sex hormones. On the other hand, the antibiotics promote a small improvement. Data compiled by Burroughs on the oral feeding of stilbestrol indicate that the carcasses averaged very close in grade to the controls and differences would not be apparent to the average person. In general, however, carcasses of stilbestrol-treated animals represented in Table 1 would be heavier, so for equal weights, the grade differential could be expected to be somewhat greater. There are reports that show that less fat and more lean tissue for hormone-treated animals of weights equal to controls. Most of the extreme cases of carcass differences have occurred where high doses of drugs have been used.

LAMBS

Most compounds studied in the fattening of cattle have been tested in lambs. Much of the work has been done in the feedlot although, as with calves, there have been tests on suckling lambs.

In the early work with implanted stilbestrol relatively large doses of 12 and 15 mgs. were tried. Results were not favorable mainly because of the so-called side effects in accentuated sex characters, prolapses of the rectum, and incidence of urinary calculi. Growth rates were increased but carcass quality frequently decreased. Subsequent studies using implants of 2 to 6 mgs. have been more favorable. Table 2 shows a sampling of published data on rates of gain increases and feed savings. Generally, improvement in growth rate and feed economy was about as good as with the larger-sized implants, and the side effects have not been significant. Carcass quality and dressing percentage still have not equalled control groups.

TABLE 2.--Summary of Experimental Comparisons of Feed Additives in Sheep Rations

Feed additive	How administered	Dose	Expt'l comparison	Gain increase	Feed saving
		Mg.	*Number*	*Percent*	*Percent*
Stilbestrol	oral	1-3	41	18.3	12.1
Do	implant	2-15	33	27.2	20.2
Do	do	3	12	27.2	19.1
Testosterone	do	20-240	10	9.8	16.8
Tranquilizers	oral	variable	12	7.2	4.2
Antibiotics	do	do	53	8.4	7.0

In tests on oral feeding of stilbestrol, the dosages have run up to about 3.5 mgs. per animal per day with the 2 mg. level being fully effective. Reports show little mention of any undesirable side effects. The carcass quality data show only a slight disadvantage for the stilbestrol-treated animals. The values shown in Table 2 for 41 lot comparisons indicate an appreciable increase in rate of gain and in feed efficiency although not as high as for implanted stilbestrol.

Of the other sex hormone products that have been tried, the principal one--testosterone--has shown a modest increase in rate of gain although much less than for stilbestrol (see Table 2). The feed savings appear to be well in line with that for stilbestrol. Limited comparisons with dienestrol implants have shown comparable results.

There have been a number of experiments with various tranquilizers. As indicated by the average figures in the table, small increases were obtained in rate of gain and in feed efficiency. It appears that lambs responded better than cattle to the use of tranquilizers.

Antibiotics, likewise, have given greater responses, percentage-wise, than have cattle in feedlots in increased gains and feed savings. It is true, however, that wide ranges exist and the individual tests do show frequent cases of negative results. Undoubtedly, reduction in enterotoxemia is an important factor in the benefits derived from the use of antibiotics in addition to the possible gain and feed advantage.

Data examined on carcass grades of lambs indicate much the same picture as for beef carcasses. For example, out of 34 comparisons from oral administration of stilbestrol, 20 showed a decrease in grade and 12 an increase. Implanted animals show a greater trend toward decrease in grade. With antibiotics and tranquilizers there appears to be little if any effect on carcass grade.

DAIRY CATTLE

The two main groups of feed additives that have attracted research interest and have been given serious consideration in practical application to dairy cattle are the hyperthyroid-producing compounds and the antibiotics.

Iodinated protein or thyroprotein, as well as synthetic l-thyroxine, when fed to milking cows, produces an increase in milk production. A few tests have been conducted with beef cows nursing calves. Results have not been impressive. Recent work on dairy cows has been summarized in Publication 714 of the National Research Council (11). The statement is made that "If thyroprotein is to be used in the stimulation of milk production, its feeding should begin about two months after lactation starts. It should continue for not longer than three to four months and should be discontinued during the last two or three months of the lactation period." Milk yields within the 3 to 4 month period may be increased 10 to 25 percent. To achieve this and to avoid excessive loss in weight in the cows, feed intake must be added as well as improved management. Feeding of the material late in lactation is not generally advised because of possible effects on viability of calves and on subsequent lactation.

Many investigations have been conducted on the use of various antibiotics in the rearing of dairy calves. While there have been contradictions in results, the main body of evidence shows first a benefit in reduced incidence of diarrhea or scours and secondly, a stimulation in rate of growth. The period of use has been studied extensively. In general, the greatest benefit appears to be derived from feeding during early life up to possibly 4 months. In experiments at Beltsville, the use of chlortetracycline during the first 90 days of life has been most beneficial in reduced incidence of scours. Any increase in rate of growth compared to controls during this period is not evident in weights of calves by the time they reach the age of 6 months or a year.

SWINE

As already mentioned, the thyrogenic compounds attracted the attention of animal nutritionists during the 1940s. In the case of swine, nearly all of the early research concerned with the feed additives treated in this report was devoted to the two groups of compounds that either enhance or retard the activity of the thyroid glands. Iodinated protein was the principal enhancing substance and thiouracil the inhibitory one tested.

In growing pigs, the iodinated protein had little effect on growth rate, feed efficiency, and carcass characteristics. Such effect as observed was usually found in the first month or two following weaning. Recently, the protein compound along with triiodothyronine has been tried in lactating sows. In one study, iodinated protein improved growth of the suckling pigs.

In the work with thiouracil and related compounds, it was found that the more promising use of the drug was in the last month prior to marketing. As is evident in Table 3, even this application did not prove beneficial, the growth rate being depressed, the feed saving of little consequence, and carcass quality unimproved. In a study at Beltsville (5) on the interaction of temperature and thiouracil feeding upon feeding and carcass characteristics it was observed that a 30° to 40° F. temperature differential modified the effect of the thiouracil. At approximately 50° F., the drug increased rate of gain, decreased backfat thickness, and increased protein content over that of controls. At a higher temperature approaching or at 90° F. the thiouracil tended to retard rate of growth while carcass fatness was not affected significantly. This work illustrates the complexity of the problem and difficulties in practical applications.

The use of other hormones of the sex group has not yielded the favorable results with swine that have been found in beef cattle. Stilbestrol, estradiol, progesterone, testosterone, and others have been tried both orally and by injection or implant. As shown in Table 3, effects on rate of gain have been nil and feed savings have been small. The reasons have not been apparent. Some years ago a study was carried out at Belts-

ville (12) on gilt pigs using a wide range of oral doses. An impressive feature of this study was the changing effects observed on estrus manifestations with increase in dosage. Whether swine are more sensitive to compounds, such as stilbestrol, and optimal dosages have not been found, remain as unanswered questions. Side effects in both males and females have been prominent in many studies.

The use of antibiotics in swine feeding presents a much more favorable picture than for hormones. Numerous reviews have been published showing benefits in terms of rate of gain, feed savings, disease level, and others. The data in Table 3 for antibiotics as a group are based on figures from Jukes (6). They represent an average for five antibiotics most used in swine feeding. The ranking in effectiveness for growth is: chlortetracycline, oxytetracycline, streptomycin, penicillin, and bacitracin. There has been a question for several years whether presently used antibiotics would lose their effectiveness of whether the disease level would be so improved as to render the antibiotics less useful. A summary compiled by J. D. Drain and W. F. Kwoek (3) on 10 years of tests at experiment stations indicated that control lots of pigs have improved in performance while chlortetracycline-treated pigs have maintained high performance. In other words, the gap between treated and untreated pigs has narrowed during the 10-year period. There have been questions as to effects on fatness of swine carcasses. However, the data do not show any appreciable increase in fatness through the use of antibiotics.

TABLE 3.--Summary of Experimental Comparisons of Feed Additives in Swine Feeding

Feed additive	How administered	Dose	Expt'l comparison	Gain increase	Feed saving
		Mg.	*Number*	*Percent*	*Percent*
Arsenicals	oral	15-45/lb.	8	2	2
Antibiotics[1]	do	5-10/lb.	325	19(35-9)	5(10-0)
Chlortetracycline[2]	do	10/lb.	87	14	4
Stilbestrol	do	0.5-2.5/lb.	4	0	3
Do	implant	3-50	12	0	2
Other sex	do	--	7	-5	9
Testosterone	oral	20-40/day	5	0	0
Iodinated protein	do	1.35-80/lb.	7	0	0
Thiouracil	do	227-1134/lb.	10	-23	1

[1] Adapted from Jukes (6), as average for chlortetracycline, oxytetracycline, streptomycin, penicillin, and bacitracin.

[2] Adapted from American Cyanamid Co., Public. (1959) Production Performance of Swine--Ten Years After Introduction of Aureomycin.

Of the other feed additives, the chief group studied in swine is the arsenicals. Research data are somewhat conflicting. Used therapeutically, arsenicals are quite effective in preventing or controlling swine dysentery. Arsanilic acid has been reported effective as an agent in reduction or prevention of selenium toxicity in growing pigs fed diets containing dangerously high levels of this element. The growth and feed savings from the use of arsenicals do not show significant trends and have been so indicated in Table 3.

In general, the few tests with tranquilizers have failed to show beneficial effects. In one case at least, reduced activity was noted with resulting reduction in feed consumption and rate of gain. A few tests with enzymes have shown some promise. Certain types of diets may be benefited more than others. Starter diets for young pigs may be improved, for example.

As with other classes of livestock, various combinations of hormones, antibiotics, arsenicals, tranquilizers, and other chemicals have been tested. A few have shown some additive effects.

The ability of estrogens administered to growing chickens to increase the fat content of the tissues was known for some time before interest was aroused in the practical application of the procedure. When diethylstilbestrol became available, Lorenz of California (8) demonstrated the feasibility of increasing the fat content of breast and leg muscle by implantation of the hormone. Numerous subsequent studies have clearly proven that hormone administration is effective in fattening and improving the finish in most classes of poultry.

Experimental results concerning the effect of hormone treatment on growth rate are not clear cut. About half of the investigators have reported some improvement, while the others found no effect. Studies at the Texas Station (2) on the effect of protein level on the growth rate indicated that the greatest response of implanted diethylstilbestrol was obtained on lower levels of protein, 14 and 16 percent. No growth response was obtained on a 21-percent diet. Similar results have been observed with turkeys at Beltsville. These findings, however, have no particular practical application since hormone treatment on the lower protein levels did not give optimum growth and feed conversion.

There is general agreement that hormone administration does not enhance feed conversion. A number of investigations show that the efficiency of feed utilization is decreased by the treatment.

Carcass analyses have shown that the increase in fat content resulting from hormone treatment is accompanied by a percentage decrease in protein, thus indicating that the live-weight gain is almost entirely due to greater deposition of fat. It has also been reported that higher cooking losses were encountered in hormonized birds, which in some cases amounted to 20 percent more than those obtained with the control groups.

Since these findings showed no economic advantages of using stilbestrol in broiler production the practice was not widely adopted, and in recent years has been essentially discontinued.

As to the use of estrogens in the specialized production of capons, the practice of which was terminated late in 1959, there was, no doubt, some economic advantage. For example, reports of experiments showed that hormonized birds were heavier, required less feed, and excelled in finish as compared to either normal males or surgical capons at 24 weeks of age.

The preponderance of experimental evidence shows that antibiotics enhance the growth and feed efficiency of chickens. By no means, however, have the experiments indicated a real advantage from antibiotic supplementation. These conflicting findings have led to numerous investigations of factors that influence antibiotic response. The general conclusion can be drawn that, under conditions of stress, that is, presence of unfavorable organisms, extremes in ambient temperature, disease, and crowding, antibiotics have proven most effective. In commercial broiler production one or more of these conditions is almost invariably present.

Studies on the interrelationship of antibiotics with other nutrients, such as the sparing effect on protein and B-vitamins, have provided some very interesting but extremely confusing information. Until sufficient knowledge is obtained concerning these relationships, they are useful as possible explanations for discrepancies in experimental results, but have no obvious practical application. An exception is the interaction between dietary calcium and antibiotics. It has been shown that low calcium enhances absorption of chlortetracycline. This has been advantageous when it is necessary to use the antibiotic at therapeutic levels.

There has been a considerable amount of work published indicating that antibiotics lose their effectiveness when used over a period of time and that the newer antibiotics

are more likely to give a response. However, an experiment recently completed at Beltsville indicated that zinc bacitracin gave as good a growth rate and feed conversion as spiramycin, an antibiotic which had not been previously used. Results of antibiotic feeding at the University of Wisconsin, covering a period from 1951 to 1954 and 1955 to 1959, were given at the 1960 meeting of the Informal Poultry Nutrition Conference. Although there was extreme variability in the results of both periods, the general trend showed about the same response for both periods.

The results of studies on the effect of antibiotics on laying hen performance have been more controversial than those with growing chicks. Some work at Beltsville has furnished a possible explanation for these contradictory reports. Our findings indicate that when a hen is laying below her genetic capacity because of stress conditions, or some subclinical infection, antibiotics may improve production. On the other hand, when a healthy bird is producing at or near her genetic capacity, antibiotic feeding is of little value. Another report at the 1960 Informal Poultry Nutrition Conference from Ohio State University, indicated that antibiotics were most effective during cold weather. Conversely, studies at our Glendale Station have shown that high-level antibiotic feeding, 50 to 100 gms. of chlortetracycline per ton of feed, consistently improved egg production during the extremely hot summer months encountered in that area. These reports are not necessarily contradictory but rather emphasize the fact that antibiotics are more effective under stress conditions.

The majority of reports on the inclusion of arsenicals in the diets of growing poultry show increased growth rate and in some cases increased efficiency of feed utilization. As in the case of antibiotics, the responses to arsenicals are variable depending upon environment and the type of diet being supplemented. Early work at Beltsville indicated that the arsenical effect differed somewhat from that of antibiotics since occasionally the two effects were additive.

Perhaps the most comprehensive study on the effect of arsenicals on growth rate is that of Milligan, et al (9) involving 13 different experiments and almost 10,000 broiler chicks. They found that when practical diets contained low levels of antibiotics it was economically advantageous to supplement with arsonic acid.

The effect of arsenicals on egg production is less clear cut, since some investigations indicate no effect and others only a small increase. A 3-year study at Beltsville failed to show any influence of arsanilic acid on egg production, whereas the work at the Glendale Station indicated that arsenicals are of some value in supporting production in hot climates.

Both arsonic and arsanilic acids have been fed at levels as high as 335 gms. per ton of feed without harmful effect. At higher levels toxicity was observed. Arsanilic acid at 0.03-percent dietary level results in increased clotting time of blood.

Reports by Texas workers in 1957 (7) indicate that a low level molybdenum supplementation (0.13 to 0.3 p.p.m.) improved chick growth on a practical diet. All other reports on the effect of molybdenum have been essentially negative. However, because of the very slight cost and on the possibility that it may be of some value, many commercial feeds are supplemented with this mineral.

At levels of 300 to 500 p.p.m. of molybdenum, toxicity was exhibited by a severe growth depression and 6,000 p.p.m. resulted in an 80-percent growth depression over the control. Feeding 0.3 p.p.m. supplementary molybdenum resulted in a substantial increase of the element in chicken blood and liver. Levels of 500 p.p.m. were toxic for the laying hen and resulted in molybdenum content in the egg of 12 p.p.m. Work at Beltsville has demonstrated an interrelationship between molybdenum and sulfate and molybdenum and copper since these two materials counteract to some extent the growth depression caused by feeding 300 and 500 p.p.m. of molybdenum.

Experimental work thus far reported on other feed additives has not shown much promise. Possibly the use of enzymes under special dietary conditions may find a useful place. The few tests with tranquilizers have not shown real benefits as yet. Nutritionists are concerned with other feed additives more properly classed as nutrients such as trace minerals, vitamins, and amino acids. Certain mineral elements are essential yet become toxic at high levels.

PROBLEMS

Some of the problems in the use of feed adjuncts from the standpoint of animal production were mentioned in the foregoing discussion. Even though many animal feeding experiments have been reported, conflicting results leave questions as to practical applications and profitableness from use in numerous cases. Obviously, the interrelations with other dietary nutrients, the feeds used in the diets, the management of the animals, the period in the life cycle, the quantities of the chemicals used, and other related factors influence the responses.

While the antibiotics probably present fewer problems than the other materials, details of the actual manner in which they function still remain in doubt. Some of the newer materials have not been studied sufficiently to judge their place and usefulness and basic information on the way in which they function in one animal species as compared to another is largely nonexistent. The combining of two or more materials to determine additive effects is complex. The statement is made frequently that it is good insurance to include a particular chemical. This may be good investment in some cases but used too indiscriminately simply adds to the feed cost.

A pressing need, especially in the case of hormones, is the development or simplification of existing methods applicable to feedstuffs, to body fluids and tissues, and to excreta in order to study the metabolic function, the ways in which the materials affect metabolism and growth, and how much or how little of the material is really needed. With such tools, it should be possible to evaluate hormonal capacities of different species and strains of animals. Out of this might well come the development through genetic means of animals that will not need supplementation through the diet or by implant.

In the case of stilbestrol, why is it that implants of 12 to 15 mgs. of the material have been the effective amount for chickens, whereas for a lamb many times larger, this is excessive? Doses of 24 and 36 mgs. are quite effective in 600-pound steers. Are the several classes of animals so different in physiological functioning as to require these differing ratios to body size? What are the checks and balances in endocrine interrelations in different species, and in different strains and lines of breeding?

A major problem area is that of naturally occurring substances in feeds. It has been known for some time that legumes, particularly the clovers and alfalfa, contain substances having estrogenic properties. The difficulties in Australia in carrying sheep on subterranean clover pastures is well known. Ohio sheep farmers have told us how they have learned to avoid prolonged use of clover pastures because of poor lamb crops. On the other side of the picture are reports pointing to beneficial effects in feeding alfalfa to fattening cattle not unlike those obtained with stilbestrol supplementation.

The Western Utilization Research and Development Division has reported the isolation of the estrogen, coumestrol, from alfalfa. While the legumes seem to show higher contents of estrogenlike substances, other plants show reactivity also. More studies are needed to identify the distribution of such compounds in plant life and the cultural practices that govern the contents. Should strains of forages be identified and developed to supply growth-promoting principles in natural forms?

Undoubtedly, many more chemicals will be synthesized and tested for use in animal production. New ones may replace some of those now being studied and used. Some new

additives may even be essential nutrients presently unrecognized and unidentified. Molybdenum is of current interest in poultry nutrition. There has been interest in the selenium toxicity problem for a number of years. Recently, selenium has become recognized as an essential element and certain areas of the country have been pointed to as producing crops so deficient in selenium as to affect livestock. In many cases specific substances are harmful in excess but are essential for animal health in lesser amounts. The hormone chemicals in many respects can be classed in this category. It is not a safe rule, however, that if some is good, more is better. Besides a better understanding of basic functions, we still need to know the optimal and safe amounts to use of a lot of nutrients that go into animal diets.

REFERENCES

1. Andrews, F.N., and J. F. Ballard. The effect of partial thyroidectomy on the fattening of steers. Proc. Am. Soc. An. Prod., 1940, 112-116.

2. Camp, A. A., J. R. Couch, and J. H. Quisenberry. Response to diethylstilbestrol injections of growing chicks fed various protein levels. Poul. Sci. 36, 171-178, 1957.

3. Drain, J. J. and W. F. Kwoek. Production performance of swine at experiment stations ten years after the introduction of Aureomycin. 10th Annual Feed Symposium, 1959. American Cyanamid Co., Agricultural Division, New York 20, N. Y.

4. Iowa State University, Ames, Iowa. Feed additives in beef cattle rations. Report AH 805.

5. Johnson, E. F., N. R. Ellis, and C. F. Winchester. The interaction of temperature and thiouracil feeding upon characteristics and feeding characteristics of pigs. Jour. An. Sci. Vol. 15, No. 1, Feb. 1956.

6. Jukes, Thomas H. Antibiotics in Nutrition, Medical Encyclopedia, Inc., 1955.

7. Kurnick, A. A., B. L. Reid, R. N. Burroughs, H. D. Stelzner, and J. R. Couch. Effect of distillers' dried solubles and molybdenum on the growing chick. Proc. Soc. Exp. Biol. and Med. 95: 353-356, 1957.

8. Lorenz, F. W. Effects of estrogens on fowl and industrial applications. Vitamin and Hormones, 12: 235-275. 1954.

9. Milligan, J. L., H. L. Wilcke, J. E. Marr, and R. M. Bethke. Arsonic acid in commercial poultry rations. Poul. Sci. 34, 794-799, 1955.

10. National Research Council. Hormonal relationships and applications in the production of meats, milk and eggs. Pub. 266, 1953.

11. National Research Council. Hormonal relationships and applications in the production of meats, milk and eggs. Pub. 714, 1959.

12. Stevenson, J. W., and N. R. Ellis. Effects of reproductive functions of diethylstilbestrol administered orally to gilts. Jour. Applied Physiology, Vol. 5, No. 9, March 1953.

USE OF CHEMICALS FOR CONTROL OF PARASITES OF FOOD ANIMALS

A. O. Foster, F. D. Enzie, and D. K. McLoughlin[1]

Some 300 species of animal organisms are economically significant parasites of food animals. They are the insidious protozoa and helminths, commonly known as internal parasites, and the ubiquitous arthropod parasites which, for convenience, are often called external parasites. In 1954 the Department estimated that internal and external parasites were about equally responsible for losses that exceeded $1 billion annually at the farm level. Developments of the intervening 6 years have indicated that the estimate was conservative. Arthropod parasites, from the standpoint of chemicals used against them, are adequately covered by other papers of this symposium. Internal parasites, or just "parasites," are, therefore, the primary subject of this discussion.

Our position with respect to parasite control is extremely acute and vulnerable. It is so because foreign chemicals are the most powerful aids available. They must yet be relied upon to roughly the same degree that immunological measures are employed for controlling pathogenic viruses, rickettsiae, and bacteria. From standpoints of other and broader alternatives to chemical control, there is little for use against parasitic diseases that is not also applicable to other diseases.

In general, problems of parasitism are intensified by shifts toward better agriculture and land usage. Changing agricultural practices, such as grassland conversion, irrigation, reclamation of barren lands, concentrated production, free movement of stock, and geographical relocations within the industry are causing ever-increasing alarm about parasite loss and a mounting urgency about combative measures. Other factors are contributory, such as better diagnosis and fuller cognizance of the economic impact of parasitic disease, more farm animals and higher inventory value per unit, and keener competition in production and marketing.

Against this background, one must weigh today's antiparasitic situation. Available chemicals, as useful as they are, are not satisfactory; even the best are quite short of the ideal. Despite great progress in the last 45 years, we still lack chemical measures for controlling about two-thirds of our injurious helminths of food animals. The situation with protozoan infections is only comparatively better. The kinds of parasitisms against which chemical agents can be used with even modest efficiency are, therefore, a minority of the important ones. Also, while not surprising, it is nevertheless unfortunate that parasites are unusually nimble at circumventing chemical attack. Chemotherapy is traditionally close to parasitology and there are few, even in this day of enlightenment, who do not recognize an enormous agricultural and human debt to progress in this field, yet ever since the classical work of Paul Erlich, populations of organisms exposed to chemicals have demonstrated their capacity to develop tolerance. Added to these considerations is the matter of toxicity, which has always been a gremlin in the development of antiparasitic agents, yet there would probably not be the pressure for alternatives to chemical control that is now manifest if events had not created a mass fear of insidious chemical poisoning and legislation had not curtailed the number and uses of available agents and, at the same time, made the cost of new developments almost prohibitive. It is well, and undoubtedly wise, that we recognize the seriousness of our situation in parasite control.

There is no question about soundness of the position on residues, yet food animals and food crops are hard hit by it, with one of the heaviest blows falling on parasite control. No defense of antiparasitics, however justifiable, can or should alter in any way or measure the requirements imposed. "Residue" legislation has caused less concern than embarrassment to scientific research. The latter is rarely confronted so clearly with an

[1] Parasitologists, respectively, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture.

instance of need for self-imposition of a course that will fulfill its functions. The paramount import of legislation may be an awakening to the fact that science must infinitely transcend any modest requirements of society.

It is inevitable that we should describe the present situation as we have. Research is geared only to the future, and respects the present and past only for what they mean to the future. Let us allow that the historian would see it differently. He would discern marked and heroic progress--progress almost of a kind to have lulled us into a position of false security. Tick fever was eradicated from cattle in this country by an unrelenting chemical attack on the vectors of the microparasite that caused the disease. Incidentally, the almost incredible story of tick fever, like that of the recent screwworm campaign, should be studied for its true significance, namely, that no iota of knowledge can be spared in developing the attack and, conversely, that no attack can succeed in the absence of an enormous body of knowledge. Such stories teach the difficult lesson of today--that knowledge is power!

Any contemplation of antiparasitics developed within the Department, including carbon tetrachloride in 1921 and tetrachloroethylene in 1925, both of which promptly became invaluable in the treatment of countless thousands of human victims of hookworm disease, for which the latter is even today the treatment of choice, reveals that most of the chemical measures against parasites of livestock that have been widely employed in this country and throughout the world were developed by the Department. Although the record makes one hesitant to name the outstanding veterinary anthelmintic, there can be little doubt in informed minds that phenothiazine must be accorded that rank. This drug combines a unique range of application, a high degree of efficiency, an unusual margin of safety, an ease and versatility of administration, and a variation of antiparasitic actions not found in any other anthelmintic.

Only yesterday, notable antiparasitic developments were on the verge of revolutionizing standards and concepts of production, performance, feed-conversion, quality, and condition of livestock and poultry. These developments, and others, indelibly established at the farm level that parasite control is an essential factor in quality and profitable production. They also prove, so far as one may judge from the acid test of experience, that notable achievement is within reach through adjuvant reliance on foreign chemicals, because most, if not all, of these materials in common use today are regarded and accepted as safe from a consumer standpoint.

It is patently unfeasible to present what should be said about antiparasitic chemicals. On this account, we have prepared, as an appendix, a "thumbnail" tabular statement of parasitisms of food animals from standpoints of chemicals now used against them, in the order of their apparent usefulness, with special reference to the fate of these chemicals, toxicity, and potential supportive or alternative measures. We may, however, look briefly into this substantial aspect by using, as an example, the notable anthelmintic to which reference has already been made, namely, phenothiazine.

Notwithstanding the Department's role in the discovery of the anthelmintic action of phenothiazine and its later contributions to knowledge of its use, what we say is taken from innumerable sources. This is inevitable, since more than 3,000 publications pertain to the antiparasitic action of this compound.

Phenothiazine has disadvantages, which must be borne in mind if we are to progress. It is comparatively expensive; therapeutic doses are bulky; some animals, notably horses and swine, are rather susceptible to intoxication by it; treated animals eliminate the breakdown products as a red dye in the urine and feces, which is alarming although harmless; the dye stains the wool of sheep (in which the drug has its greatest overall usefulness); and discolors milk. It is also deficient in desired efficacy against some parasites against which it is hopefully employed, such as trichostrongyles of sheep and many gastrointestinal nematodes of cattle. Moreover, there has been no adequate determination of efficient physical forms of the chemical; its pharmacological actions; its remote and long-term effects, or net economic advantages of systems of low-level and free-choice

administration. The drug does not remove all worms, of course, and it is apparently therapeutically ineffective in animals whose digestive function is seriously impaired. It is little wonder that there is concern over practical failures of phenothiazine when one considers the wide and almost exclusive reliance on it as a panacea for gastrointestinal parasitism! Notwithstanding its shortcomings, we are nevertheless confident, despite imperfect knowledge of the balance sheet, that the chemical presents no consumer hazard.

The fate of orally administered phenothiazine has been studied in man, cattle, sheep, horses, pigs, dogs, rabbits, and rats. Derivatives have been identified in the alimentary tract, feces, blood, urine, and aqueous humor. At least eight catabolic or excretion products have been identified, namely, unchanged phenothiazine, leucophenothiazone, potassium leucophenothiazone sulfate, phenothiazone, leucothionol, leucothionol glucuronide, thionol, and phenothiazine sulfoxide. There is also a compound in the urine of treated animals which is said to yield the parent substance upon acidification, and a polypeptide conjugate in the urine of cattle. Information on the fate of phenothiazine in any one species of animal is incomplete, but the aggregate information on all species shows that detoxication is relatively complicated, with somewhat differing mechanisms in different species of animals but without much distortion or breakdown of the parent molecule. Derivatives vary also with age and dose rate within a single species. Kind of food may exert some influence, at least quantitatively. Thionol is apparently the end result of the detoxication mechanisms in all animals. This is a conspicuous red dye, which is at least helpful in determining the rate of elimination. So far as determined, phenothiazine and its derivatives are completely eliminated from the animal body in about 4 days. The intermediates, incidentally, although not far removed from phenothiazine, are evidently not toxic to either the host or its parasites. The only exception seems to be phenothiazine sulfoxide which has anthelmintic properties and is responsible for occasional photosensitization keratitis in cattle, and possibly also for the same phenomenon in sheep and goats.

As of today, legislation has made antiparasitics suspect, and some even outlawed, but this is scarcely the major concern in the area of protozoan and helminthic parasites. Safety has long been the definitive criterion, not only for reasons that are evident, but as prudent policy in conformity with responsibilities of Federal regulatory agencies. Indeed, safety to consumers as a factor in the standardization of antiparasitics anticipated present legislation in the instances of arsenic, lead, fluorine, and cadmium, all uses of which were adjudged as of the time to be without consumer hazard. This is of record. However tenuous the grounds, it could be argued that antiparasitics, as recommended for use today, are as safe from a consumer standpoint as our public water supply.

Problems are inordinately real and obvious; they have been heralded by previous discussion. Most significant is that of finding the road back to the scientific method in the development of control measures. A few investigators have emphasized the seemingly irrefutable fact that knowledge of mechanism of drug action, physiology of parasites, biology of parasitisms, and especially the delicate adjustments thereof, are the basis on which to build. Chemotherapy arose during what might naively be called the "golden age" of biochemistry when many chemists turned their attentions to living things, to the effects of chemicals on them, to the chemistry and fate of foreign chemicals in the animal body and, significantly, to the concept and study of selectively toxic action of chemicals on closely associated but differing species of living organisms. Selective toxicity is the epitome of problems that want for elucidation in chemical attack on living things. The road back to this concept is not readily apparent.

Problems of antiparasitics are comprehensive. Immediate problems are evident--all referable to basic criteria of safety, efficiency, use, and cost. But the real problem is parasitism--the true substance of parasitology--a way of life that characterizes the majority of kinds and numbers of living things. While animal parasitology has traditional roots in three or four phyla of the animal kingdom, modern parasitology has become increasingly oriented about parasitism as a biological phenomenon. What do parasites do? Why are they parasites? How do they live? These are the questions being asked. We know little, for example, about the role of parasites as vectors and reservoirs of pathogens, as agents and

incitants in the expression of disease, including tumors, as stress factors, and as agents in nutritional interference. We know less about the way parasites live, their physiology, special enzymes, nutritional requirements, and hormones. If we intend to confront the real enemy on a rational basis, we cannot long neglect such knowledge or serious study of the fundamental processes of life as they obtain in this common and complex phenomenon. Only upon a backlog of solid knowledge along these lines can we effectively expand the use of chemicals in parasite control. Serendipity will undoubtedly play its continued role in this area as it proverbially has in all science through all time, but this moment may well mark the last when we want for even a single example in all chemotherapy of an agent that was rationally developed. Selectively toxic chemicals are destined increasingly to be "made-to-order."

The design of acceptable and effective control measures requires more than the selective, physiologic, basic approach. If we trust reason, observation, and experience, effective control is predicated on a certain fullness of knowledge of total problems. This means, as it meant for cattle fever and screwworms, knowledge about speciation, life-cycle, parasite-host relationships, bionomics, immunity, geographical distribution, epizootiology, sources of infection, and means of transmission. In short, the design of any attack on parasitic or other disease can ill afford to leave any stone unturned in the development of all facets of the problem.

A third, significant aspect of need concerns development of knowledge of the place of chemicals in parasite control. It is a paradox that those who are closest to problems of chemical control seem most commonly obliged to emphasize that this approach is never the whole solution, and rarely the best, to any problem. A primary urgency in parasite control is thorough inquiry into potential means of minimizing or circumventing reliance on foreign chemicals. At the very minimum, there should be full exploration of efficient means of doing the job with the least amount of chemical and of measures that obviate the direct use of chemicals in or on food animals. Any true evaluation of the place of chemicals in parasite control must scientifically depend upon a weighing of all approaches, including alternatives to chemical measures. If today's guess is of significance, it can only stress that total problem knowledge is the cornerstone of the best control measure. There is usually only one best solution to a problem. To this end, there must be extensive investigation of immunological phenomena, of parasite pathogens, predators, and hyperparasites, of the delicate associations and adjustments of parasites at all stages of their development and in their varied environments. This want of knowledge of parasitism and parasitic diseases clearly means that parasitology is today a discipline of dimensions and depth undreamed a decade ago.

We have emphasized basic biology of parasitism as the foundation for rational development and expanded use of antiparasitic chemicals, reconnaissance of total problems as the foundation for best usage, and intensive inquiry into alternatives to chemical measures as the foundation for properly evaluating the place of chemicals in parasite control. Without these things, further effort toward improved chemical controls is indefensible if not doomed to failure.

One other problem must be mentioned. It is the question of unbiased evaluation of new agents. During recent years, industry has become especially active in the development of antiparasitics. It has done what appears to have been a notable service.

Medicated feeds have unquestionably proved to be the key to prevention of many devastating diseases of food animals, especially poultry. Many products are competitively marketed and, for some purposes, the number of products has mushroomed on a scale which seems quite unwarranted by the experimental background.

The grower has traditionally looked to the Department for advice on the product that is best for him. Today, he is confused by the bombardment. Is he not entitled to the one best answer to his question?

And what about industry? It has top capability and has demonstrated unrealized potential of service to the livestock industry. It wants, so far as our experience suggests, exactly what the grower wants. For different reasons, both genuinely want independent evaluations. Such research, moreover, can scarcely be in conflict with the responsibilities of regulatory agencies. We submit that this is a major and primary research obligation that cannot be sidestepped without disservice alike to farmers, manufacturers, regulatory agencies, research organizations, and the public. It is simply a question of getting what is best for agriculture!

There is, we sense, a growing consciousness of the necessity for unbiased evaluation before consumer use of chemicals, especially drugs. If true, this is most significant; it means that we shall inevitably proceed toward a better solution of the problem of chemicals in agriculture. If not true, the urgency of the job ahead to find this solution gives us little time. Agriculture will suffer without safe, efficient chemicals; and unbiased evaluation may be the only means of assuring such chemicals. It is unfortunate that current events have resulted in some curtailment of industrial endeavor in anti-parasitic chemicals. Present policies may serve less than they should. There is room for thought because the interests and welfare of all depends upon implicit trust in the unprejudiced findings of research.

In this discussion, we have emphasized the importance of parasitism as a biological phenomenon, the economic significance of parasitic diseases of food animals, and our heavy reliance upon chemicals as adjuvants in their control; also the apparent safety of such chemicals and the urgency, as of today, of broadening our development and use of them. It has been equally emphasized that the answers to today's problems, which now threaten further progress, are to be found largely in more knowledge about parasites and parasitism. Finally, there is need for unbiased evaluation of new agents as a solution to a difficult problem, as a service to all interests, and as a means of bolstering the confidence of all in the objectivity of research.

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Anaplasmosis Anaplasma marginale	Tetracyclines	1 to 5 mg/lb daily for 5 days i.v.	Some complications arise from alteration of intestinal flora.	Peak blood concentration quickly reached. Reduced to trace amounts in 24 hrs. Primary excretion, in urine and feces.	Diagnosis (C.F.) and slaughter; vector control; cross-immunization.
	Neoarsphenamine	5 to 6 gm i.v.	Effects similar to those of arsenic poisoning. Temporary renal impairment, hepatitis and sometimes nervous manifestations.	Concentration falls rapidly. Excretion in urine begins promptly. Chief excretion by bile in feces. Slow and incomplete.	
	Sodium cacodylate	45 grains in 10 ml i.v.	Renal impairment.	Part excreted unchanged in urine; greater part undergoes reduction to cacodylic acid and inorganic arsenic.	
Coccidiosis Eimeria spp.	Sulfonamides: Sulfamethazine (4,6-dimethyl-2-sulfanilamido-pyrimidine)	60 mg/lb daily for 3 to 4 days or in chopped hay or grain at 1 gm/lb of feed for 3 to 4 days. Capsule; or sodium salt i.v.	Acute: Temporary blindness, muscular tremors and weakness. Anorexia and diarrhea. Chronic: Renal impairment.	Peak blood concentration within 12 hrs. Readily distributed to all tissues. Undergo acetylation, extent varies with compound. Excretion primarily in urine, with exception of poorly absorbed sulfonamides which are primarily excreted in the feces. Small amounts excreted in bile, pancreatic, gastric and intestinal juices, saliva and milk. Most rapidly excreted in first few hours after reaching bloodstream.	Symptomatic treatment of diarrhea. Portable pens for calves. Segregation by age groups. Separation of young stock from manure accumulations.
	Sulfamerazine (2-sulfanilamido-4-methylpyrimidine)	60 mg/lb daily for 3 to 4 days. Capsule; or sodium salt i.v.			
	Sulfaquinoxaline (N^1-[2-quinoxalyl] sulfanilamide)	60 mg/lb daily for 3 to 5 days. Capsule			
	Phthalylsulfathiazole (2-[N^4-phthalylsulfanilamido]-thiazole)	60 mg/lb daily for 3 to 5 days. Capsule			
Large stomach worm Haemonchus spp.	Phenothiazine (thiodiphenylamine)	Therapeutic: 10 gm/cwt (max. 60gm) Drench or capsule Prophylactic: 1 to 2 gm/day Free-choice in mineral or other supplement.		Converted to various degradation products and excreted in feces, urine and milk. (See text.) Eliminated within 4 days.	Free-choice phenothiazine. Good feeding.

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Large stomach worm--Contd.	Copper sulfate	1 ml/lb body weight (1% sol.) Drench	Negligible; fatal dose 200 to 400 gm.	Copper stored principally in liver; but, ordinarily, 90% excreted in feces.	
	Copper sulfate and nicotine sulfate	1 ml/lb body weight (1 oz. Black Leaf 40/gal. 1% $CuSO_4$ sol.) Drench	Slightly more toxic than copper sulfate solution.	Copper: (see above). Nicotine: metabolized rapidly.	
	Hexachloroethane	50 to 100 gm Drench	Inappetence, lethargy, incoordination, narcotization, death (associated with low nutritional plane).	Eliminated promptly through bile.	
	Tetrachlorethylene	5 ml/cwt (in 1 to 2 parts mineral oil) Drench (pre-dose 60 ml 10% $NaHCO_3$ sol.)	Dizziness, bloat, coughing, choking.	Low absorbability.	
Medium stomach worm	Phenothiazine	20 gm/cwt (max.60 gm) (See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)
Ostertagia spp.	Tetrachlorethylene	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)	
Small hairworm	Phenothiazine	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)
Trichostrongylus axei et al.	Hexachloroethane	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)	
Large roundworm Neoascaris vitulorum	Piperazine (diethylenediamine)	10 gm/cwt Capsule or drench	Negligible.	Eliminated promptly, primarily (90%) in urine.	
Hookworm	Phenothiazine	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)
Bunostomum phlebotomum	Toluene (methylbenzene)	10 ml/cwt (add 10% emuls. agent) Drench (fast; pre-dose 60 ml 10% $NaHCO_3$ sol.)	Incoordination; inappetence.	Converted to hippuric acid and elminated in urine.	
Nodular worm	Phenothiazine	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)
Oesophagostomum radiatum	Piperazine	(See Neoascaris.)	(See Neoascaris.)	(See Neoascaris.)	

CATTLE--Continued

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Tapeworms *Moniezia* spp.	Lead arsenate	0.5 to 2.0 gm Capsule	Lethal dose of lead 0.1 to 0.2 gm/lb body weight.	About 60% of absorbed lead in bones, 25% in liver; eliminated largely in feces, some in milk. Arsenic excreted in urine, feces, and milk.	
	Bis(2-hydroxy-5-chlorophenyl)methane	6 gm/cwt Drench	Negligible.		
Liver fluke	Hexachloroethane	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus.)	Molluscacides; draining; fencing; filling
Fasciola hepatica and Rumen flukes *Paramphistomum* spp.	Carbon tetrachloride	5 to 10 ml Capsule	Muscular tremors, diarrhea, prostration, convulsions. Hepatic necrosis. Lethal dose 10 to 20 ml.	Eliminated primarily through lungs; some excreted in urine.	
Lungworms *Dictyocaulus viviparus*	Cyanacethydrazide	Parenteral: 15 mg/kg (max. 5 gm) Subc. inj. (daily 1 to 3 days) Oral: 17.5 mg/kg (max. 4.5 gm) Drench or in feed (daily 1 to 3 days)	Depression; inappetence; mild convulsions; decreased milk yield.	Rapidly eliminated; small amount excreted in milk (none after 24 hrs.).	Avoid low, damp pastures. Remove infected animals to dry lot. Good feeding.

SHEEP

Coccidiosis *Eimeria* spp.	Sulfaguanidine (Sulfanilylguanidine monohydrate)	Prophylactic: 3 gm/lamb/day Capsule or in feed (0.2%) Therapeutic: 0.45% in feed	(See Coccidiosis--Cattle.)	(See Coccidiosis--Cattle.)	Clean and dry pens. Pasture rotation. Avoid over-crowding.
	Succinylsulfathiazole (2-[N^4-succinyl-sulfanilamido]-thiazole)	1 gm/day In feed	(See Coccidiosis--Cattle.)	(See Coccidiosis--Cattle.)	
	Sulfamethazine	0.1 gm/kg Daily for 5 days Capsule	(See Coccidiosis--Cattle.)	(See Coccidiosis--Cattle.)	

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Large stomach worm Haemonchus contortus	Phenothiazine	Therapeutic: Adults 20 to 40 gm Lambs 10 to 20 gm Drench or capsule Prophylactic: 0.5 to 1.0 gm/day Free-choice 1:9 in mineral mixture	Negligible.	(See Cattle.)	Free-choice phenothiazine. Good feeding.
	Copper sulfate	(See Haemonchus--cattle.)	Negligible.	(See Haemonchus--cattle.)	
	Copper sulfate and nicotine sulfate	(See Haemonchus--cattle.)	Somewhat more toxic than copper sulfate solution. Not well tolerated by animals in poor condition.	(See Haemonchus--cattle.)	
	Carbon tetrachloride	1 to 2 ml Capsule or drench (in 2 to 5 parts mineral oil)	Occasional death.	(See Fasciola--Cattle.)	
	Tetrachlorethylene	2.5 to 5 ml (in 2 parts min.oil) Drench (pre-dose 10 ml 1% $CuSO_4$ sol.)	(See Haemonchus--Cattle.)	(See Haemonchus--Cattle)	
	Hexachloroethane	30 gm Drench	(See Haemonchus--Cattle.)	(See Haemonchus--Cattle.)	
Medium stomach worms Ostertagia spp.	Phenothiazine	(See Haemonchus.)	Negligible.	(See Haemonchus--Cattle.)	Free-choice phenothiazine. Good feeding.
	Copper sulfate and nicotine sulfate	(See Haemonchus.)	(See Haemonchus.)	(See Haemonchus--Cattle.)	
Stomach hairworm Trichostrongylus axei	Phenothiazine	(See Haemonchus.)	Negligible.	(See Haemonchus--Cattle.)	Free-choice phenothiazine. Good feeding.
	Copper sulfate and nicotine sulfate	(See Haemonchus--Cattle.)	(See Haemonchus.)	(See Haemonchus--Cattle.)	
	Tetrachlorethylene	(See Haemonchus)	(See Haemonchus--Cattle.)	(See Haemonchus--Cattle.)	
Hookworm Bunostomum trigonocephalum	Phenothiazine	(See Haemonchus.)	Negligible.	(See Haemonchus--Cattle.)	Do.
	Copper sulfate and nicotine sulfate	(See Haemonchus--Cattle.)	(See Haemonchus.)	(See Haemonchus--Cattle.)	
	Carbon tetrachloride	(See Haemonchus.)	Occasional death.	(See Fasciola--Cattle.)	
	Tetrachlorethylene	(See Haemonchus.)	(See Haemonchus--Cattle.)	(See Haemonchus--Cattle.)	

SHEEP--Continued

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Small intestinal hairworms Trichostrongylus colubriformus et al.	Phenothiazine	(See Haemonchus.)	Negligible.	(See Haemonchus--Cattle.)	Free-choice phenothiazine. Good feeding.
	Copper sulfate and nicotine sulfate	(See Haemonchus--Cattle.)	(See Haemonchus.)	(See Haemonchus--Cattle.)	
	Tetrachlorethylene	(See Haemonchus.)	(See Haemonchus--Cattle.)	(See Haemonchus--Cattle.)	
Cooperias Cooperia curticei et al.	Carbon tetrachloride	(See Haemonchus.)	Occasional death.	(See Fasciola--Cattle.)	Do.
	Phenothiazine	Prophylactic: (See Haemonchus.)	Negligible.	(See Haemonchus--Cattle.)	
	Bephenium(e) [hydroxynaphthoate]*	75 mg/lb Drench or capsule			
Thread-necked worms Nematodirus spp.	Phenothiazine	Prophylactic: (See Haemonchus.)	Negligible.	(See Haemonchus--Cattle.)	Do.
	Bephenium(e) [hydroxynaphthoate]*	(See Cooperias.)			
Nodular worms Oesophagostomum spp.	Phenothiazine	(See Haemonchus.)	Negligible.	(See Haemonchus--Cattle.)	Do.
	Piperazine	100 mg/lb Drench or capsule	Negligible.	(See Neoascaris--Cattle.)	
Large-mouthed bowel worms Chabertia ovina	Phenothiazine	(See Haemonchus.)	Negligible.	(See Haemonchus--Cattle.)	Do.
Tapeworms Moniezia spp.	Lead arsenate	0.5 to 1.0 gm Capsule	3 to 6 gm tolerated by animals in good condition. (See Moniezia--Cattle.)	(See Moniezia--Cattle.)	
	Copper sulfate	(See Haemonchus--Cattle.)	Negligible.	(See Haemonchus--Cattle.)	
	Copper sulfate and nicotine sulfate	(See Haemonchus--Cattle.)	(See Haemonchus.)	(See Haemonchus--Cattle.)	
	Bis(2-hydroxy-5-chlorophenyl)methane	0.1 gm/lb Drench	Negligible.		
Liver fluke Fasciola hepatica Rumen flukes Cotylophoron spp. et al.	Carbon tetrachloride	(See Haemonchus.)	Occasional death.	(See Fasciola--Cattle.)	(See Fasciola--Cattle.)
	Hexachloroethane	(See Haemonchus.)	(See Haemonchus--Cattle.)	(See Haemonchus--Cattle.)	

(e) Experimental.
* Benzyldimethyl-2-phenoxyethyl-ammonium (3-hydroxy-2-naphthoate).

SHEEP--Continued

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Thread lungworm Dictyocaulus filaria	Cyanacethydrazide	Parenteral: 15 mg/kg (max. 1 gm) Subc. inj. (daily 1 to 3 days) Oral: 17.5 mg/kg (max. 1.5 gm) Drench or in feed (daily 1 to 3 days)	(See Dictyocaulus--Cattle.)	(See Dictyocaulus--Cattle.)	(See Dictyocaulus--Cattle.)
Hair lungworm Muellerius capillaris Red lungworm Protostrongylus rufescens	Emetine hydrochloride	0.15 ml/lb (1% sol.) I.m. inj. (repeat in 48 hrs.)	May lose wool.	20% of dose may accumulate in liver; relatively high concentration may persist for more than a month; very little in skeletal muscle. Very slowly destroyed.	
Arterial worm Elaeophora schneideri	Piperazine(e)	25 gm Drench or capsule	Negligible.	(See Neoascaris--Cattle.)	
	Antimony potassium tartrate	35 to 40 ml (1% in glucose sol.) I.m. inj. (weekly 7 to 8 wks.)			

(e) Experimental.

SWINE

Eperythrozoonosis Eperythrozoon sp.	Neoarsphenamine	1 to 2 gm/100 lbs i.v.	(See Anaplasmosis--Cattle.)	(See Anaplasmosis--Cattle.)	
Large roundworm Ascaris lumbricoides	Piperazine	50 to 60 mg/lb In feed or water	Negligible.	Eliminated promptly, primarily (90%) in urine.	Sanitation system of management.
	Hygromycin	12M units/ton feed to 100 lbs body wt.	Occasional deafness; may depress appetite in swine over 100 lbs.	Promptly eliminated--no tissue residue.	Sanitation system of management.
	Sodium fluoride	1% of dry, ground feed for 1 day (Approx. 0.13 gm/lb body wt.)	Vomition; soft feces.	Only insignificant traces of fluorine in soft tissues after 1 week--no consumer hazard.	
	Cadmiun oxide	0.015% of feed for 3 days.	Vomition.	Small amount of cadmium retained in soft tissues, insignificant after 30 days.	

SWINE--Continued

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Large roundworm--Contd.	Cadmium anthranilate	0.044% of feed for 3 days.	Vomition.		
Nodular worms	Piperazine	(See Ascaris.)	Negligible.	(See Ascaris.)	(See Ascaris.)
Oesophagostomum spp.	Hygromycin	(See Ascaris.)	(See Ascaris.)	(See Ascaris.)	
	Phenothiazine	0.15 gm/lb (max. 30 gm) In feed	Photosensitization. CNS disturbances.	(See Haemonchus--Cattle.)	
Lungworms Metastrongylus spp. Choerostrongylus pudendotectus	Cyanacethydrazide	(See Dictyocaulus--Sheep.)	(See Dictyocaulus--Cattle.)	(See Dictyocaulus--Cattle.)	(See Ascaris and Dictyocaulus--Cattle.)
Stomach worms	Sodium fluoride	(See Ascaris.)	(See Ascaris.)	(See Ascaris.)	(See Ascaris.)
Ascarops strongylina Physocephalus sexalatus	Carbon disulfide	4.5 ml/cwt stomach tube	Vomition; dizziness; prostration.	85 to 90% metabolized and eliminated in urine.	
Whipworms Trichuris suis	Hygromycin	(See Ascaris.)	(See Ascaris.)	(See Ascaris.)	(See Ascaris.)

CHICKENS (PROTOZOA)

Coccidiosis Eimeria spp.	Nicarbazin (equimolar complex of 4,4'-dinitrocarbanilide and 2-hydroxy-4,6 dimethyl pyrimidine)	0.008 to 0.0125% In feed Fed continuously	Reduced feed intake and reduced weight gain; ataxia; incoordination; liver, kidney and pulmonary damage. Decreased egg production and interference with shell pigmentation.	Splits into its components. DCN absorbed more rapidly, but disappears more slowly from tissue. Only trace amounts left 4 days after withdrawal of drug.	Clean, dry and uncaked litter. Removal of droppings. Avoid overcrowding. Age segregation. Immunization.
	3,5-dinitrobenzamide	0.025% in feed. Fed continuously. (Used in combination with acetyl-(para-nitrophenyl) sulfanilamide and 3-nitro-4-hydroxyphenyl-arsonic acid.			
	Glycarbylamide (4-5 imidazole-dicarboxamide)	Broilers: 0.006% in feed Fed continuously. Replacement flocks: 0.002% in feed Fed continuously.		Only trace amounts absorbed by blood when fed at normal levels. Eliminated 48 hrs. after withdrawal of drug.	

CHICKENS (PROTOZOA)--Continued

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Coccidiosis--Contd.	Arsenosobenzene (phenyl arsenoxide)	0.002% in feed Fed continuously			
	Bithionol (2,2'-thiobis[4,6-dichlorophenol])	0.05% in feed Fed continuously (Used in combination with methiotriazamine.).		No residue detectable 48 to 72 hrs. after withdrawal of drug.	
	Methiotriazamine (4,6-diamino-1-[4-methylmercaptophenyl]-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride)	0.01% in feed. Fed continuously. (Used in combination with bithionol.)		No tissue residue 48 to 72 hrs. after withdrawal of drug.	
	Nitrofurazone (5-nitro-2-furaldehyde semicarbazone)	0.0055% in feed Fed continuously	Depressed growth, curled toe paralysis, damage to lungs, kidney and bursa of Fabricius.	Reduction of nitro group, presumably to hydroxylamine believed to be initial metabolic process. Small amount of reduced compound excreted in urine.	
	Sulfaquinoxaline	Prophylactic: Intermittent, 0.05% in feed Continuous, 0.0125% in feed Therapeutic: 0.1% in feed or 0.04% in water	Decreased weight gain. Damage to liver, kidney, bone marrow and thyroid. Extreme levels cause fall in egg production.	Readily absorbed from digestive tract. Acetylation not extensive in chickens. Blood concentration decreases slowly after drug withdrawal. Traces present up to 112 hrs. after withdrawal.	
	Sulfamethazine	Therapeutic: 0.44% in feed or 0.2% in water for 3 to 5 days	Interference with weight gain, prolonged blood clotting time. Hyperplasia of seminiferous tubules. Precocious development of comb and wattles. Decreased egg production.		

TURKEYS (PROTOZOA)

Coccidiosis *Eimeria* spp.	Sulfaquinoxaline	Prophylactic: 0.025% in water, intermittently, or 0.0175% in feed, continuously Therapeutic: 0.025% in water, or 0.05% in feed Intermittent schedule	(See Coccidiosis--Chickens.)	(See Coccidiosis--Checkens.)	(See Coccidiosis--Chickens.)

TURKEYS (PROTOZOA)--Continued

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Coccidiosis--Contd.	Sulfamerazine	0.4% in feed for 4 to 6 days, or 0.2% in water on intermittent schedule			
	Sulfamethazine	Do.	(See Coccidiosis--Chickens.)		
Histomoniasis Histomonas meleagridis	Nithiazide (1-ethyl-3-[5-nitro-2-thiazolyl]-urea)	Prophylactic: 0.025 to 0.035% in feed Fed continuously Therapeutic: 0.02% in water 7 to 10 days followed by prophylactic level	Delayed sexual maturity. Depressed egg weight.	Not detectable in plasma 3 to 6 hrs. after withdrawal of drug. Some concentration in egg yolk. No residue in liver, kidney muscle or fat.	Range rotation. Segregation of chickens and turkeys. Age segregation. Control of cecal worm.
	Acetylamino nitrothiazole (2-acetylamino-5-nitrothiazole)	Prophylactic: 0.015 to 0.025% in feed Fed continuously Therapeutic: 0.05% in feed 14 days or longer, followed by prophylactic medication	Some growth retardation. Decreased egg production.		
	Furazolidone (N-[5-nitro-2-furfurylidine]-3-amino-2-oxazolidone)	Prophylactic: 0.011% in feed Fed continuously Therapeutic: 0.011 to 0.0165% in feed for 14 days or longer, followed by prophylactic medication	Decreased food consumption. May delay sexual maturity. Can cause decrease in number of spermatozoa.	Very little absorbed by blood-stream, and quickly eliminated.	
	4-nitrophenylarsonic acid	0.025% in feed Fed continuously 0.019% in water continuously	May cause decreased growth and decreased egg production.		
Hexamitiasis Hexamita meleagridis	Furazolidone	(See Histomoniasis.)	(See Histomoniasis.)	(See Histomoniasis.)	Age segregation. Separation of carrier (recovered) stock. Dry, clean litter. Keep sick birds warm and comfortable.
	Nithiazide	(See Histomoniasis.)			

CHICKENS AND TURKEYS (HELMINTHS)

Parasite	Chemicals used	Administration	Effects of excessive or prolonged dosage	Fate in body	Supportive control measures
Large roundworm *Ascaridia galli*	Piperazine	100 mg/bird In feed or water	Nil	Eliminated promptly.	Temporary runs. Good diet, especially high in vitamin A.
	Nicotine (sulfate)	50 mg/bird In feed for 2 to 3 days	Decreased feed consumption Lowered egg production.	4 to 25% excreted unchanged through kidneys; most of remainder detoxified in liver.	
	Tetrachlorethylene	0.5 to 1.0 ml/bird Capsule or intubation	(See Haemonchus--Cattle.)	Low absorbability; promptly eliminated.	
	Carbon tetrachloride	(See tetrachlorethylene)	Muscular tremors, diarrhea; hepatic necrosis.		
Cecal worm *Heterakis gallinae*	Phenothiazine	0.5 to 1.0 gm/bird Capsule or consumed in feed within 8 hrs. 0.74% of feed for 2 to 3 days	Nil	(See Haemonchus--Cattle.)	Temporary runs. Age segregation.
Tapeworms *Raillietina cesticillus* *Choanotaenia infundibulum* *Davainea proglottina* etc.	Di-n-butyltin dilaurate	125 mg/bird In feed	Liver necrosis at toxic but sublethal dosages. LD_{50} 700 to 900 mg/kg.	Tin largely excreted in urine; 20 to 25% retained in skin, 5% in liver.	Control of invertebrate intermediate hosts.
	Hexachlorophene (Bis[2-hydroxy-3,5, 6-trichlorophenyl] methane)	15 to 20 mg/lb Capsule or in feed	Decreased egg production (30%)		
Gapeworm *Syngamus trachea*	Antimony barium tartrate	30 gm/8 cu.ft. chamber Inhalation of dust-laden air in tightly closed chamber for 25 to 30 minutes.			(See Heterakis.)

CHEMISTRY OF PESTICIDES IN SOILS

L. A. Dean[1]

The ultimate fate of pesticides added to soils depends upon physical, chemical, or biological processes operating singly or in sequence. Literature reviews reveal that pesticidal behavior cannot be predicted from studies with a single soil or from a single pesticide on several soils. This discussion deals with certain principles of soil chemistry as they are related to behavior of organic substances added to soils. The purpose is to examine the types of information required for a satisfactory prediction of pesticidal behavior in soils.

As early as 1907 (13), U. S. Department of Agriculture scientists were interested in the occurrence of organic toxic substances in soil. In fact, the theory was then advanced that such substances contributed to the unproductivity of certain soils. A poor soil was found to contain the phytotoxic substance dihydroxy-stearic acid.

Soils consist of mineral and organic matter, water and air combined in three phases: Solid, liquid, and gaseous. Soil chemistry furnishes the basis of our knowledge of the composition and the interrelations of these components. For the purpose of this discussion perhaps the most important characteristic of soils is the differences among them. Every farm consists of several kinds of soils or soil types, and throughout the world there must be hundreds of thousands of soils. Individual areas of a soil type are three-dimensional bodies having length, breadth, and depth. Differences between neighboring soils may be large or small. They reflect changes in such factors as topography, drainage, or parent material. Regional soil differences are generally greater than those of local associations of soil types. Most frequently these differences reflect the distribution of climate and living organisms. For convenience soil types are grouped in respect to common features thus bringing out relationships between soils and their management in relation to agriculture. The soils of the arid Western States can be contrasted with those of the humid Southeast. The former are neutral or alkaline in reaction, very low in organic matter and clay minerals of the montmorillonite expanding-lattice types predominate. The latter are acid, contain appreciable quantities of organic matter, and in the clay fraction kaolin and the hydrous oxides of iron and aluminum predominate. These are examples of the contrasts in properties that may exist between two soil zones.

THE PERSISTENCE OF ORGANIC SUBSTANCES IN SOIL

One common feature of soils is the accumulation or persistence of varying quantities of organic residues. For the most part these residues originate from plant and animal tissues returned to the soil, however, in recent years an ever-increasing variety of organic materials are being added to soils by man. Many factors have been studied in relation to the persistence of organic residues in soil including climate, oxygen supply, and chemical and physical properties of soils.

The climatic factors of temperature and rainfall have a strong influence on the rate of decomposition of organic matter. As an example of this influence of climate, an experiment conducted at 11 locations in the Sierra Nevada mountains is cited (8). The annual decomposition of alfalfa under these different climates is shown in Figure 1.

At first sight the decomposition curve seems erratic, but actually, it shows that the effects of rainfall and temperature are closely recorded. The first three points show the influence of sharply increasing rainfall. Points 3 to 7 show a decreasing decomposition rate with decreasing temperature but constant rainfall. The remaining points reflect a temperature-rainfall interaction.

---

[1]Research Investigations Leader, Soil and Water Conservation Research Division, Agricultural Research Service, U. S. Department of Agriculture, Clemson, S. C.

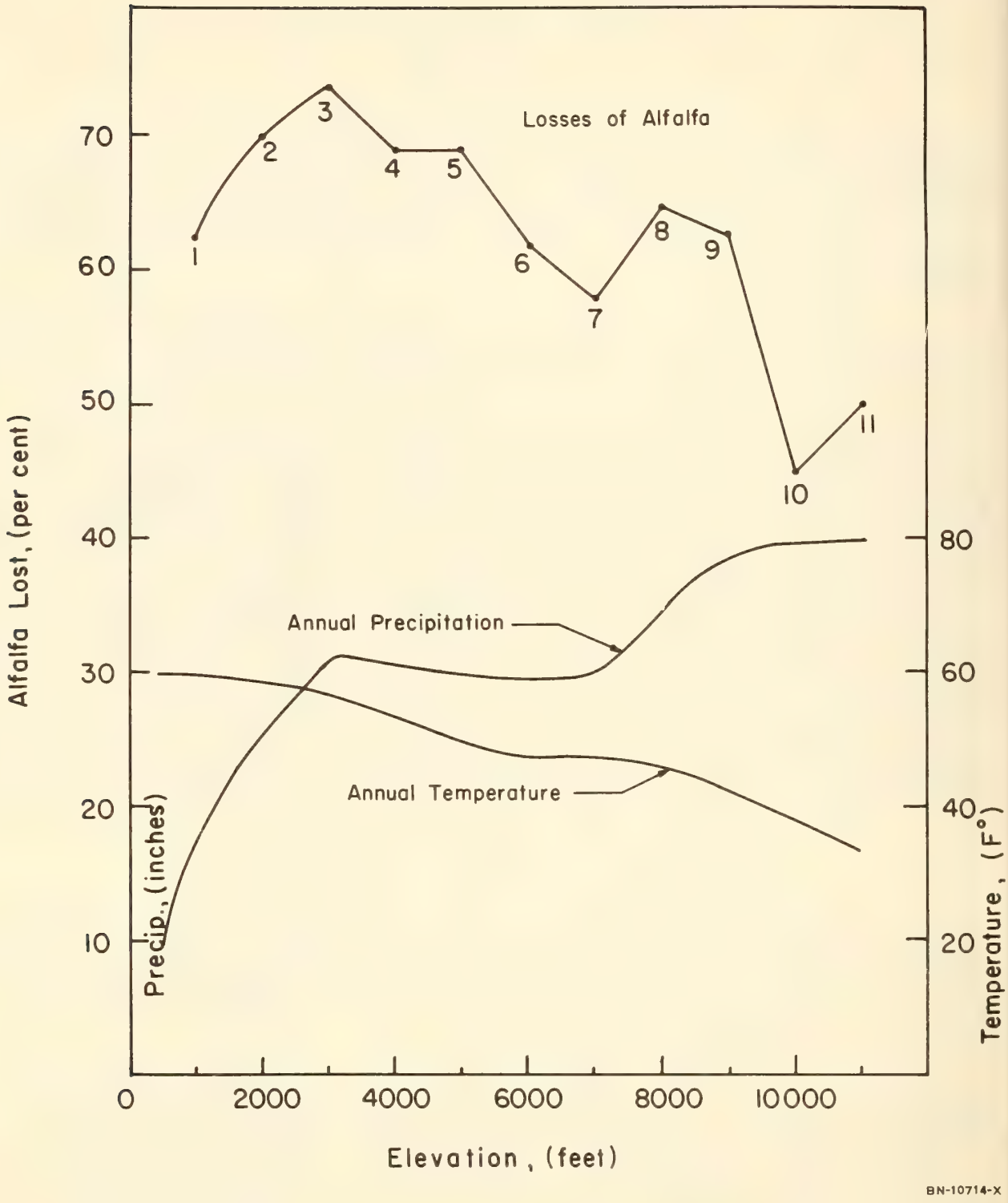


Figure 1.--Annual losses of alfalfa in the Sierra Nevada Mountains.

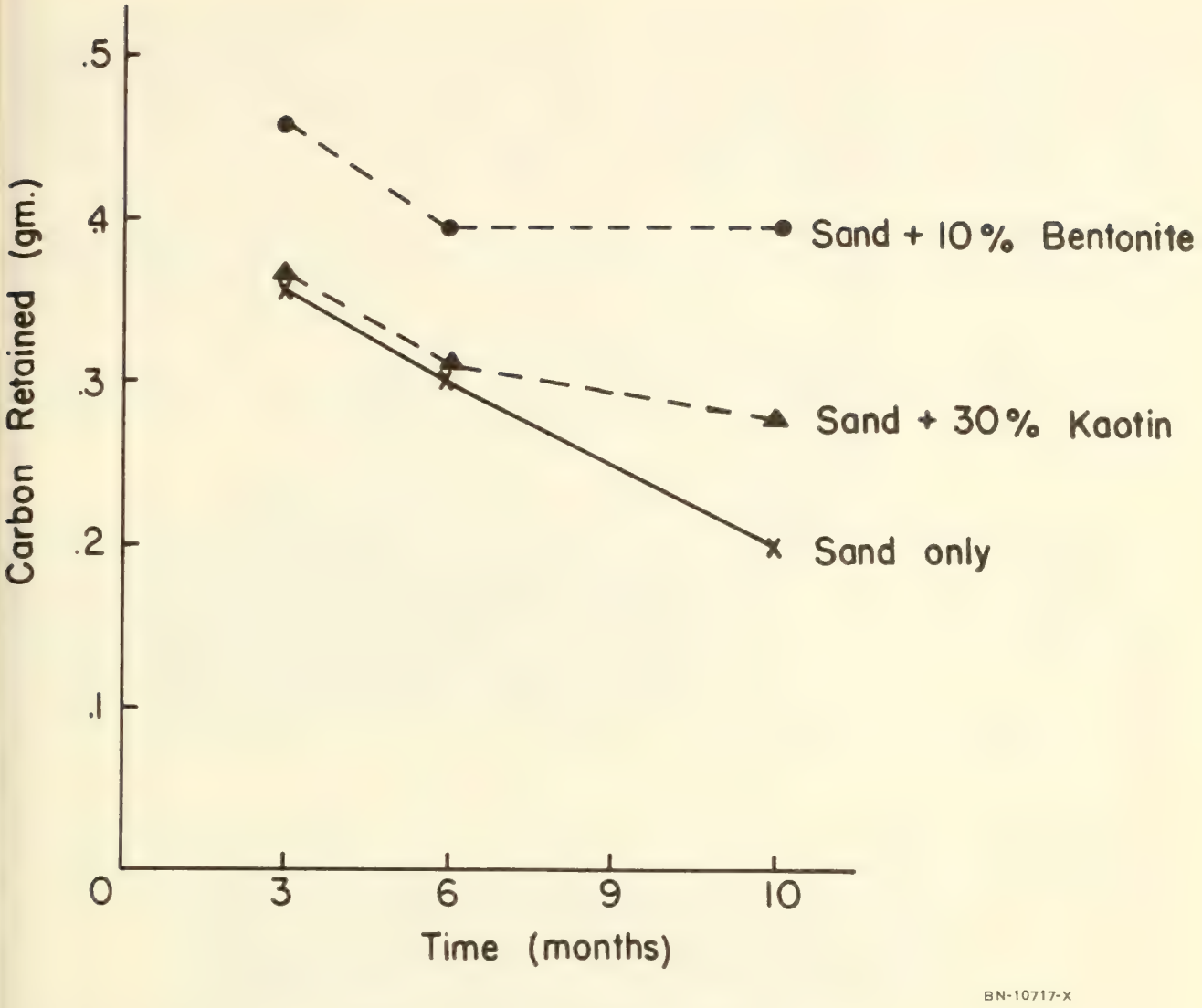


Figure 2.--Effect of clay on retention of organic carbon.

A well-known observation is that under similar climatic, age, degree of aeration, and cropping conditions the organic matter content of soils increases with their clay content. This suggests that the clay may in some way contribute to the persistence of organic matter in soils. Data from a controlled laboratory experiment by Allison, et al. (1) have been selected (Figure 2) to illustrate the influence of clay on the persistence of organic substances in soils. In this experiment 1 gm. lots of carbon as soybean plants were incubated with 100 gms. of sand or sand-clay mixtures, and the persistence of the carbon measured. The data clearly show that the introduction of clay into the system had a marked influence on the persistence of the organic carbon. Further, clay of the bentonite type (montmorillonite) appeared to be much more effective in this respect than kaolin.

When considering the persistence of organic substance in soils, the tacit assumption is that the agent for decomposition is a biological process or its attendant enzymatic activity. However, for the purposes of this discussion the possibility that chemical or photochemical reactions in the absence of biological activity may alter organic molecules in contact with soils should not be precluded. The postulation of such reactions in soils is an old one, but there is only a limited amount of supporting evidence. For example, Dean and Dean (2) demonstrated the decomposition of citric acid in contact with soil to carbon dioxide and acetone. Presumably the soil supplied a necessary catalyst.

The foregoing discussion has dealt with some of the factors which contribute to the persistence of organic carbon of living origin with the purpose of pointing out the marked influence of climate and soil factors. Obviously, the prediction of the fate of organic substances in a specific soil at a given location will require a knowledge of both climate and soil. Although our knowledge is much less extensive there is ample evidence to show that prediction of the fate of the chemicals classed as organic pesticides is dependent upon similar if not the same circumstances.

Studies were reported by Foster, et al. (5) on the accumulation of insecticides in field soils at Beltsville, Md., State College, Miss., and New Brunswick, N. J. In this instance there were three soils, three climates, and nine insecticides involved. The tendency for accumulation was greatest at Beltsville and least at State College. The relative persistence of the nine insecticides also varied with location. It is not known to what extent these differences were caused by soil or climatic changes. One generalization coming from these experiments was that many years will be required for the accumulation of sufficient evidence for accurate prediction of insecticidal persistence in soils.

The high tendency for DDT to persist and accumulate in soils is well known. A number of years ago an experiment was conducted at Beltsville which demonstrates the differential influence of soils on decomposition rate of DDT. Radioactive p,p^1-DDT was incorporated in 18 representative soils at a normal rate of application. After a 6-month period of moist incubation the soils were extracted with acetone, and the amount of radioactivity recovered from each soil measured. The loss of radioactivity (C^{14}) varied from 0 to 25 percent. An attempt was made to relate the degree of this loss to soil properties. No correlation was found with soil texture or organic carbon content and recovery, but a significant negative correlation was found with soil pH, although, there was considerable inconsistency between soils. There were five soils with a pH greater than seven, three showed a 25 percent loss of radioactivity.

It seems evident that a satisfactory prediction of the influence of soil conditions on the instability of organic molecules will require a knowledge of the biological or chemical reactions which accomplish the actual molecular alteration. Another aspect is the reactions between soils and organic molecules which will inhibit these reactions.

SOIL REACTIONS STABILIZING ORGANIC MOLECULES

Many of the organic compounds added to soils are not particularily resistant to decomposition yet they appear to have a degree of stability in the soil. The organic phosphorus compounds that accumulate in soils are a well-known example. Several hypotheses have been advanced to account for this lack of ready decomposition. A low level of microbiological activity in soil usually is a first consideration. The chemical approach deals with insolubility either through the formation of insoluble salts with polyvalent ions or the adsorption by clays and organic complexes. Molecular forces, hydrogen bonding, polar molecules, and basic amino groups are probably all involved.

Soils high in clay have been shown to be the most retentive of organic matter. This observation has lead to the belief that some sort of an organic-inorganic interaction takes place in soils. In recent years the reactions between pure organic compounds and the clay montmorillonite have been subjected to detailed study. Gieseking (6) showed that complex organic cations such as methylene blue or brucine are strongly absorbed by the montmorillonite-beidellite-montronite clay minerals giving rise to increased c-lattice spacings. Increasing the amount of complex-cation added increased the spacing until a maximum was reached. These results were interpreted as showing that a portion of the complex cations were absorbed within the variable spacings of the minerals. Subsequently Hendricks (7) showed that the organic cations are held to the surface of silicate layers by both Coulomb forces between ions and van der Waals attraction of the molecules to the surface.

A brief review of the nature of the clay mineral montmorillonite affords a further insight into the absorption of complex organic cations. The silicate clay minerals including montmorillonite are described as having a layer-lattice structure in which one molecule is not distinct from another, but the minerals have a repeating pattern occurring in layers and building a platelike structure. The structure of montmorillonite is shown schematically in Figures 3 and 4. Each crystal layer consists of an aluminum sheet with a silica sheet above and below. The layer thickness is fixed. However, the spacing between layers varies between the limits of 3 and 24 A. Normally the interlayer spaces are occupied by water and inorganic cations such as H^+ or Ca^{++}. When montmorillonite-organic cation complexes are formed these are replaced and the lattice spacing altered (see Figure 4).

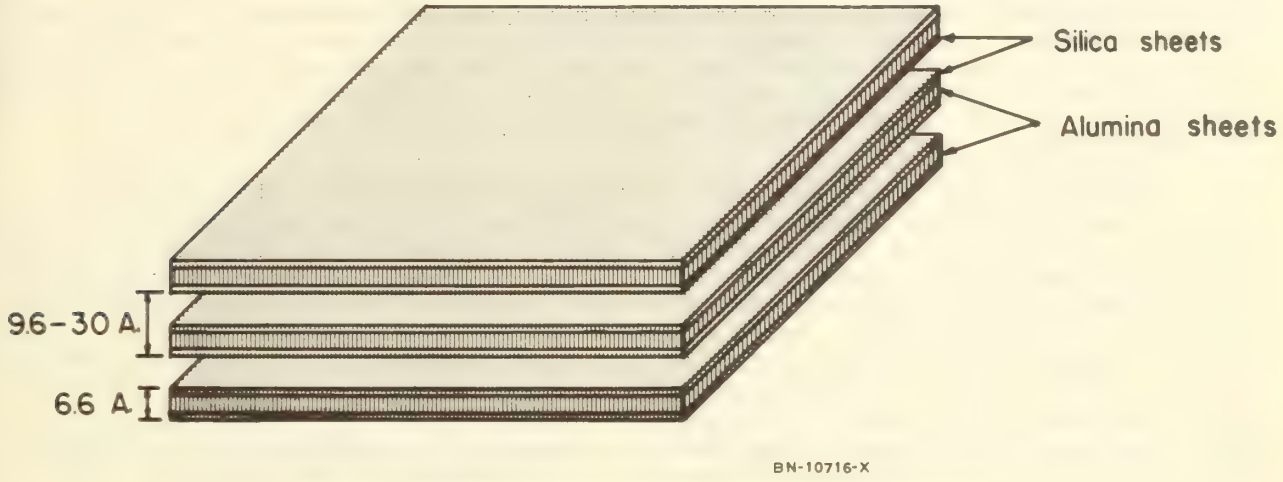


Figure 3.--Schematic representation of the structure of montmorillonite.

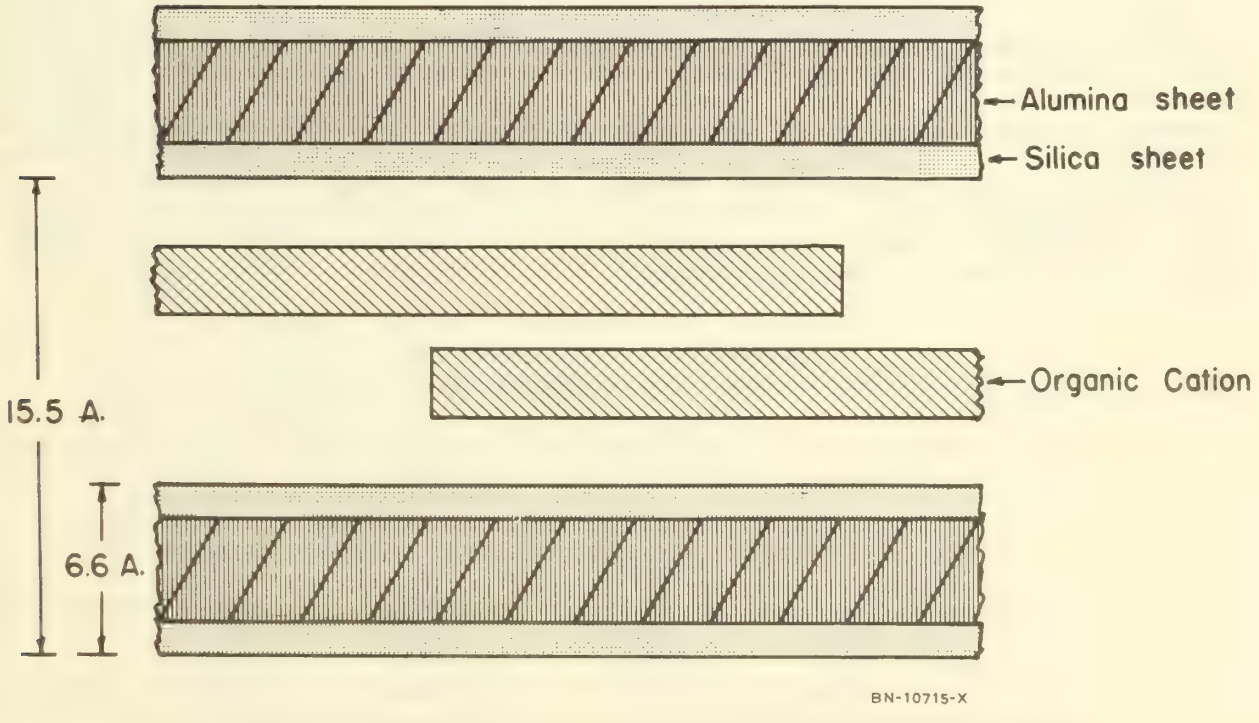


Figure 4.--Schematic representation showing molecular ions between silicate layers of montmorillonite.

Having established that complexes between large organic cations and montmorillonite are readily formed raises the question: Does the formation of such complexes change the stability of the organic molecules themselves? Ensminger and Gieseking (3) noted that protein-montmorillonite complexes prepared for laboratory study showed only a slight tendency to putrefy. Subsequent studies (4) demonstrated that the absorption of albumen and hemoglobin by montmorillonitic clays interfered with the enzymatic hydrolysis of these proteins. Further investigations were conducted by Pinck and coworkers (9) on the mechanism and extent of the decomposition of protein-montmorillonite complexes by microorganisms. Table I (9,p.117) shows the characteristics of the protein-montmorillonite complexes used and their decomposition. The low-protein complexes, 10 percent, had a 15 A c-spacing which suggests that the protein between the crystal lattices was a mono- layer. Only 20 percent of the protein in these systems was decomposed in 4 weeks and the interlayer spacing remained essentially unaffected indicating that the protein decomposed was limited to that absorbed on the external surfaces. On the other hand, the complexes having 50-percent protein had a 30 A c-spacing suggesting two or more layers of protein between the crystal lattices. These complexes decomposed readily. Apparently the proteolytic enzymes were not able to penetrate the layers having only a 15 A c-spacing, but if the spacing is large enough the enzymes are able to enter and decomposition proceeds.

The adsorption of biologically active substances by clays or soils raises the additional question: Has the bioactivity of the molecule been impaired? Studies on antibiotics in soils by Pinck and coworkers (10,11) are of interest in this respect. The 10 antibiotics used may be conveniently divided into 3 groups based upon their chemical and physical properties, namely; strongly basic, amphoteric, and acidic or neutral. The strongly basic and amphoteric types formed antibiotic-montmorillonite complexes. The former expanded the crystal lattice to 4.4 A and the latter, 7.6 A corresponding to mono- and di- layers, respectively. The antimicrobial properties of these complexes were measured by bioassay. The complexes containing strongly basic antibiotics exhibited no antimicrobial properties, whereas complexed amphoteric antibiotics could be released from the clay minerals by means of a suitable buffer and thereby regain their bactericidal activity.

The foregoing discussion has dealt with a single absorption reaction, its mechanism, and consequences. The unique feature being the expansion of crystal lattices which can be closely followed by x-ray defraction techniques. But, many soils contain no montmorillite, and the adsorption of certain organic molecules also occurs. These adsorption reactions involve the external surfaces rather than the surfaces of the inner layers of crystals, and it has not been possible to define the mechanism or products exactly. Complex organic anions do not spread the crystal lattice of montmorillonite, but they are strongly adsorbed by the same crystals (12). The adsorption of sodium polymethacrylate is an example, and in this instance an anion exchange reaction was presumed. The classifying of soils, in respect to the types of adsorption reactions that they will support with organic molecules, appears to be an important prerequisite to predicting the fate of pesticides in soils.

TABLE 1.--Characteristics of protein-montmorillonite complexes and their decomposition in 4 weeks (9).

Protein in complex		Protein-carbon evolved as CO_2	c-Spacing	
Kind	Percent		Before decomposition	After decomposition
		Percent	*A.*	*A.*
Gelatin	54.8	82.0	29.2	12.7
Do	10.6	18.4	15.0	14.6
Egg albumen	53.8	86.4	30.0	11.8
Do	9.4	17.4	15.4	15.0

SUMMARY-DISCUSSION

The significance of any organic molecule in contact with a soil depends upon the reactions or transformations that may take place in this new environment. Of most specific interest are: (1) The potentiality for breakdown whether biological, chemical, or physical, and the nature of the decomposition products, (2) the reactivity with soil constituents, (3) the retention in active or inactive forms, and (4) the movement either by leaching or volitalization. From the outset the purpose of this discussion has been to point out that the fate of this molecule depends not only on its properties, but also upon those of the soil and its physical environment. Soils have been shown to vary widely in chemical composition, properties, and physical environment. Examples have been cited to show how these changes in conditions may alter the behavior of organic molecules from soil to soil. To predict the fate of a given organic molecular species in a given soil will require, therefore, the integration of adequate knowledge pertaining to both.

LITERATURE CITED

(1) Allison, F. E., Sherman, M. S., and Pinck, L. A. 1949. Maintenance of Soil Organic Matter: 1. Inorganic Soil Colloid as a Factor in Retention of Carbon During the Formation of Humus. Soil Sci. 68: 463-478.

(2) Dean, L. A., and Dean, A. L. 1929. Decomposition of Citric Acid by Soil. Soil Sci. 28: 281-287.

(3) Ensminger, L. E., and Gieseking, J. E. 1939. The Absorption of Proteins by Montmorillonitic Clays. Soil Sci. 48: 467-474.

(4) Ensminger, L. E., and Gieseking, J. E. 1942. Resistance of Clay-absorbed Proteins to Proteolytic Hydrolysis. Soil Sci. 53: 205-209.

(5) Foster, A. C., Boswell, V. R., Chisholm, R. D., Carter, R. H., Gilpin, G. L., Pepper, B. B., Anderson, W. S., and Geiger M. 1956. Some Effects of Insecticide Spray Accumulations in Soil on Crop Plants. U. S. Dept. Agr., Tech. Bul. 1149. 36 pp.

(6) Gieseking, J. E., 1939. The Mechanism of Cation Exchange in the Montmorillonite-beidellite-nontronite Type of Clay Minerals. Soil Sci. 47: 1-13.

(7) Hendricks, S. B. 1941. Base Exchange of the Clay Mineral Montmorillonite for Organic Cations and its Dependence Upon Adsorption Due to van der Walls Forces. Jour. Phys. Chem. 45: 65-81.

(8) Jenny, H., Gessel, S. P., and Bingham, F. T. 1949. Comparative Study of Decomposition Rates of Organic Matter in Temperate and Tropical Regions. Soil Sci. 68: 419-432.

(9) Pinck, L. A., Dyal, R. S., and Allison, F. E. 1954. Protein-montmorillonite Complexes, Their Preparation and the Effects of Soil Microorganisms on Their Decomposition. Soil Sci. 72: 109-118.

(10) Pinck, L. A., Holton, W. F., and Allison, F. E. Antibiotics in Soils: I. Physicochemical Studies of Antibotic-clay Complexes. Soil Sci. [In press]

(11) Pinck, L. A., Soulides, D. A., and Allison, F. E. Antibiotics in Soils: II. Extent and Mechanism of Release. Soil Sci. [In press]

(12) Ruehrwein, R. A., and Ward, D. W. 1952. Mechanism of Clay Aggregation by Polyelectrolytes. Soil Sci. 73: 485-492.

(13) Schreiner, O., and Reed, H. S. 1907. Certain Organic Constituents of Soils in Relation to Soil Fertility. U. S. Dept. Agr., Bur. Soils Bul. No. 47. 52 pp.

THE DEVELOPMENT OF EQUIPMENT IN THE APPLICATION OF AGRICULTURAL CHEMICALS

Walter M. Carleton, L. A. Liljedahl, Frank Irons, O. K. Hedden, and R. D. Brazee[1]

INTRODUCTION

The effectiveness of any program to reduce chemical residues on plants and animals as well as the cost of applying those chemicals will be greatly affected by the kind of equipment used and the skill with which it is operated. This is true whether the application is from the air, by ground machines, or under the soil surface.

The problem of applying pesticides, if such are to be used, is to place the minimum required amount of chemical where needed with the least amount possible going elsewhere due to waste or drift. The application must be made with equipment which is both economical and practical. For example, some special operations might be accomplished by hand or with hand tools, but the operation would not be practical enough to be used even in research.

Much progress has been made in pesticide application equipment in the last 25 years, and the USDA agricultural engineers can rightfully claim a proper share of credit. However, the methods of applying dusts and sprays are still relatively costly and crude and we, in the pest control business, in order to assure control, have used the simple expedience of applying more of these pesticides than the minimum amount actually needed to control the pests. This has run up the cost and increased the residue hazards.

According to estimates only 10 to 20 percent of the material applied as dust by commercial equipment is deposited on the plant surfaces in such a manner as to be effective. The older methods of shaking a sack of dust over the plant or cranking a knapsack duster, both commonly used on tobacco farms, are even less efficient. Little is known of the efficiency of deposition of sprays or fogs on both animals and plants.

The efficiency of pesticide application is of considerable economic importance. The annual losses to agriculture resulting from disease, insects, and weeds have been estimated at more than $7,000,000,000. The annual cost of chemicals used for pest control is estimated at more than $500,000,000. Improved equipment giving a more accurate control of application rates and a more uniform application might well reduce the chemicals needed for a given application by 5 to 10 percent thus resulting in savings of $25,000,000 to $50,000,000 annually. Further, more accurately controlled and uniform application of chemicals for pest control would materially aid in solving the problem of excessive residues.

OPTIMUM PEST CONTROL ---- ASSUMPTIONS

- The ideal solution to reduction of chemical residues is elimination of the need for chemicals. This may come about by biological methods such as the fantastically successful screwworm eradication campaign. This method seems to offer the ultimate in efficiency. Another method for further elimination of chemicals is the application of pathogens (virus, bacterial, and fungus diseases) to control insect pests. The possibilities of this method of control through the introduction of selective diseases without harming the plant stirs the imagination. Yet we know little of means for depositing the bacteria or the virus and fungus most effectively.

[1]Assistant Director, Agricultural Engineering Research Division, ARS, USDA; Assistant to Acting Chief, and Agricultural Engineers, respectively; Crop Production Engineering Research Branch, Agricultural Engineering Research Division, ARS, USDA.

- If chemicals are to be used, only the minimum amount for optimum control should be used. To apply this optimum amount will necessitate an intimate knowledge of the characteristics of the plant, the insect, the disease, the particle characteristics, and the forces affecting deposition.

- If chemicals are to be used, application of the right kind of chemical must be made in the right place, at the right time, in the correct amount, and properly distributed for the needs.

PROBLEM NEEDS

Application of pesticides is a subject concerned with several disciplines. However, many of the physical and engineering aspects can be considered in isolation from entomology and crop production, providing these aspects are treated in general terms.

As stated in the introductory remarks, the usefulness of pesticide application is greatly affected by the equipment used. This is true whether the application is from the air, by ground machines, or under the soil surface.

The primary purpose of basic agricultural engineering research on pesticide application is to gain information on fundamental principles and equipment requirements which ultimately will be made available to manufacturers. Agricultural engineers also have an obligation to help implement the research of entomologists, crop and soil scientists, and economists. This is a continuing and in fact expanding need because of the rapidly changing circumstances resulting from changing crops, new cultural practices, new pests, new pesticides, and new materials of construction. We must emphasize that the existence of a generally acceptable and widely used commercial machine for a particular farm operation is not proof that there is no need for further engineering research on this operation. It would be just as reasonable to argue that there is no need for further research in crop genetics because widely accepted varieties of wheat, corn, and cotton are available.

The objectives of applied engineering research are to develop new and improved equipment and methods for more accurately directed and more uniform application of all types of pesticides, liquid, dust, granular, or biologic, to all important crops and animals. The results of such research will:

(1) Reduce the pesticide residue hazard to man, animals, and beneficial insects by reducing the application rates required.

(2) Reduce the danger of damage to crops or wildlife on adjacent areas.

(3) Improve the control of pests.

(4) Reduce the cost to farmers.

In order to attain these results we must recognize the axiom that the application of an economical minimum amount of chemical over large areas will require the division of the pesticide material into very small, although not necessarily uniform particles.

An examination of need shows that equipment must fulfill application requirements for at least the following situations:

1. On plant surfaces, which includes:
   (a) run-off spraying
   (b) low-volume spraying
   (c) dusting
   (d) granular materials
2. On the soil surface
3. Under the soil surface--gaseous and volatile materials
4. Other locations, such as on animals

Plant Surfaces. Although much work has been directed toward better application of pesticides to crop plants, very little effort has been made to more specific placement on the plant. Cooperative research by engineers and crops scientists may lead the way to material reductions in pesticide requirements by showing that the material is wholly or primarily effective on specific locations on the plant. One example is the development of granular DDT for placement in the whorls of the corn plant. Similarly a report from North Carolina researchers states that:[2]

> "The results indicate that commercial hornworm damage can be prevented with about one-fifth the recommended rate of insecticide if treatments are directed to the upper five or six leaves at the time the oldest larvae are in the third and fourth instar . . . . . Insecticide residues can be reduced considerably over those attained with grower treatments with no appreciable increase in insect damage."

Pesticide application requirements (on plant surfaces) may be differentiated according to the needs on the plant surface.

Overall discontinuous application. For herbicides which are translocated in the plant or for insects which bite and chew, it is only necessary to have a distribution of the material so that sufficient quantities are made available to bring about the required reaction. This may be either a relatively few large particles or a larger number of smaller particles reasonably well spread over the plant surface. It may be that application to the upper or to the lower surface may be of most benefit for a specific insect.

Overall continuous application. For disease control or for sucking insects the requirement may be that the material be distributed almost continuously, although in a very thin layer, over the plant surface. For example, one can calculate the necessary minimum size of particles and the distribution of those particles on a leaf to prevent the entrance of a disease spore of known dimensions. The requirements for 100 percent coverage can be very exacting and most likely unattainable in either a commercial or an experimental machine, but the requirements can be established as a goal to shoot for.

On the Soil Surface. Increasing amounts of herbicides are being placed on the soil surface to control weeds, either as pre-emergence or as post-emergence sprays. The required amounts, the method of application, and the kind of material depend upon soil and plant conditions which must be determined and defined. Again, concurrent and cooperative research by engineers and crop scientists is necessary for best progress.

Sub-surface application. In some cases the pesticide, such as a herbicide or a nematocide, may be placed under the soil. The optimum effect with the minimum amount will come about when the material is placed at the proper depth and distributed in such a manner as to come in contact with the desired pest. In addition, a pesticide such as a volatile liquid herbicide must be properly covered to obtain maximum results with minimum losses to the atmosphere.

Other applications. Specialized applications such as on animals may require additional precautions such as protecting the animal's eyes and preventing excessive inhalation.

BRIEF HISTORY OF EQUIPMENT PROGRESS TO DATE

Although thousands of inventions and developments have been recorded, the following list shows typical examples indicative of the progress.

1883	Hand sprayer
1887	Traction sprayer
1900	Gas engine power sprayer
	Engine duster

[2] North Carolina Tobacco Reprint Series No. 65, May 15, 1959.

1911 Pressure controls and air chamber pressure smoothing
1916 Adjustable sprayer
1922 Aerial dusting
1925 Air blast sprayer for orchards
1930 Aerial spraying
1935 All-steel equipment
1937 High air volume, medium velocity air blast sprayer
1938 First study of air blast field sprayers
1944 Introduction of low volume hydraulic sprayer
1948 Self-propelled high clearance sprayers
1954 Granular applicators

PRESENT STATE OF ART AND SCIENCE

Comparison of application methods. It would be helpful to be able to compare the biological effectiveness of different application methods and the physical performance of the various machines. Biological differences might then be identified with the physical differences which in turn might be common to a number of methods and/or machines. If an important effect is found to be general, a principle could be established which could be utilized in other situations.

Some indication of the availability of an insecticide may be obtained by measuring the spray-deposit or its distribution on a target area.

However, the biological effect of an insecticide, for example, is dependent upon the distribution of the insecticide on or in the insects, and this is rarely measured. We only infer that the insecticide is available whenever the insects die and vice versa.

Deposit measurement. Progress in measurement of spray deposits has been steady, but our present knowledge is by no means adequate. Most progress has been made in mass distribution in chemicals. In 1909 Riley developed equipment in which a nozzle being tested sprayed dye solution at a sheet of paper through a slot in a moving curtain. This sheet of paper then presented a 2 dimensional picture of the mass distribution of spray from the nozzle. The next major step was not made until 1947 when Barger, et al., described a corrugated device to divide spray from a nozzle into narrow bands and measured the amount intercepted in each band. This method has had widespread use ever since. However, it is a laboratory device and such tests do not necessarily indicate with any reliability the distribution of spray material that may occur in the field. Generally there is not sufficient volume in the spray material used to enable one to use these devices in the field. Weick developed a system using sensitive balances in the field to weigh the deposit directly. This equipment was adversely affected by dew deposit and wind. Davis and Eliott devised a method for making quick visual estimates from deposits which were caught on dye cards. This method is widely used in forest spraying. Miller and Isler developed a technique in which slides are laid out in an array in the field. The sprayer being tested deposits a dye solution which is washed off each individual slide and the optical density of the wash water is measured photoelectrically to provide data on general mass distribution in the field. Akkeson and Yates refined this technique by substituting a flame spectrophotometer in place of photoelectric dye measurements. Accurate measurements can be made with this equipment from a variety of salts. Hansen developed a method for the use of fluorescent chemicals to detect the presence of spray drift great distances from spraying operations. Liljedahl and Strait then developed a technique for using fluorescent chemicals for quantitative measurements of spray distribution which is fast and moderately accurate.

Less work has been done on means of measuring chemical distribution on the plant or in the soil. As an improvement over the usual direct titration, Ban and Carleton showed that polarographic techniques could be conveniently used with trace chemicals added to the spray, to greatly increase speed of measurements. Autoradiographs of the plants or leaves

have been made after spraying with material containing radiosotopes. Fluorescent chemicals have been used to measure distribution on plants as well as on and under the soil. Many of these techniques yield only qualitative or quantitative information, but one should not discount techniques because they yield only one kind of information since some information is considerably better than no information at all. However, we should not be satisfied until we have techniques for making both fast and accurate measurements on the plant and in the soil.

Particle size measurements and particle size distribution description are even more difficult and correspondingly little has been done in this field. Most work done on this problem has been by direct microscopic particle sizing and counting. This is a tedious and expensive procedure and is subject to error as well, both in sizing and in sampling. The earliest improvement over this method was developed by Tippler who used nozzles being tested to spray molten wax at a temperature which had the correct viscosity and surface tension. The spray formed was directed into a cold chamber where it froze and was collected. The wax particles formed were then sieved and counted. Elutriation in a liquid column, similar to methods used for soil particle analysis, could also be used to find particle size distribution. This technique is fast, but has the disadvantage that the results with molten wax may not always be comparable with the liquid which will be sprayed in practice, and it is of course only a laboratory technique.

Adler et. al. then developed equipment based on the photoelectric equipment used in a wirephoto machine which was in turn adapted to agricultural sprays by English researchers, Courshee and Trickett. With this equipment a photograph is taken of the droplets which have been sampled and the negative is scanned with a spot of light. A photocell opposite the light registers electric impulses of various lengths, which size and count drops electronically. This information on distribution of chord lengths which have been scanned can be converted, using suitable mathematical formulas, into drop size distribution. The equipment is limited to measuring drop sizes in any range of 1 to 20, but by photographing the droplet sample at various degrees of enlargement a wider range of droplet sizes can be recorded. This technique provides information very quickly but requires elaborate equipment. To the best of our knowledge, only 5 such installations are available in the world today.

Progress in Deposit Specification. Very little is recorded on research in deposit specifications. Work by the USDA during World War II indicated that there was an optimum size of aerosol for use in some insecticide sprays. Work by the Forest Service indicates that certain drop size yield the best results for some type of forest insect spraying work. Research at North Carolina, referred to elsewhere in this paper, showed that by directing the spray correctly on tabacco, effective insect control could be obtained with greatly reduced dosages.

Progress on Deposit Control from Ground Equipment. The earliest work in control of deposits was in the use of liquid sprays in place of dust. Average particle sizes of dust are much smaller than for sprays and have a wider variability in particle distribution as well. However, spray did not gain widespread adoption until a development of low gallonage, low-pressure sprayers. The next development in deposit control was the use of automatic controls to control pressure in spraying equipment. This was followed by adjustable spray guns which were used in orchard spraying. Research by Irons on the distribution of dust from duster blowers resulted in much improved dust distribution from field equipment. In 1948 the work of Barger et al. made possible maximum uniformity of spray patterns by correct spray nozzle placement for ground equipment.

About that same time Lovely and Collins developed photographic techniques to study the over-all spray pattern surrounding crops being sprayed, to provide a qualitative trial and error method of optimizing spray nozzle placement. In 1956 work by Cox et al. on cornborer control equipment resulted in the use of granular insecticide applied above the corn to take advantage of the natural funnel of the corn whorls in directing the granular insecticide to the point on the corn plant where the insect most frequently fed. This is the best specific deposit control which is in use today.

Control and prediction of droplet size. It is possible to predict the average drop size which will be produced by several nozzles used in agricultural work. Such formulas have been developed from dimensional analysis and empirical experiments, and appear to be accurate over the range of variables which are encountered in ground agricultural spraying equipment. For example, the following formula applies to fan nozzles in still air. It is not known whether it applies to aircraft spraying:

$$D = (4.4 \pm 0.15)\left(\frac{Q_1}{\theta}\right)^{1/3} \gamma^{1/3} \rho^{1/6} p^{-1/2}$$

where D = mass median diameter, Q_1 = nozzle discharge, θ = fan angle, γ = surface tension, ρ = liquid density, and p = liquid pressure. Similar formulas have been developed for prediction of average drop size from hollow cone nozzles, for example: $D = (2.12 Q_1 \gamma)^{1/3} (\pi \sin \beta)^{-1/3} \rho^{1/6} p^{-1/2}$ where β = the cone half angle.

A great deal of work has been done on predicting drop size from air atomizers with a corresponding variety of formulas available. The following formula, one of the earliest developed by Nukiyama and Tanasawa, still seems to fit as well as any:

$$D_{32} = 585 \left(\frac{\gamma}{V_{rel}^2 \rho_1}\right)^{0.5} + 597 \left(\frac{\mu_1}{\sqrt{\gamma \rho_1}}\right)^{0.45} \left(1000 \frac{Q_1}{Q_a}\right)^{1.5}$$

where D_{32} = surface mean diameter, V_{rel} = relative velocity of liquid and air, Q_a = air discharge, and μ_1 = liquid viscosity. However, the formula is completely empirical, has little rational basis, and is not even dimensionally consistent.

Formation of droplets from spinning disks at low flow rates is well understood. Under these conditions spinning disks yield nearly homogeneous sprays and may be usable in the field, although capacities are very limited. At high capacities we get heterogeneous sprays and the mechanism of droplet formation, though not completely understood, is probably similar to that of hydraulic nozzles.

From the previous discussion it appears we can do a fair job of predicting average drop size from various nozzles under certain conditions. However, we do not know whether the standard deviation of the drop size distribution is related to any variables, and generally the standard deviation has been believed to be uncontrollable, except for the fact that extreme upper and lower limits on the drop size usually exist.

Fan nozzles have been developed for agricultural use with both tapered and uniform distribution patterns for use side by side or alone respectively. Field studies have determined the nozzle configurations which give results which are reasonably satisfactory at present for spraying nonrow crops as well as preemergence surface spray, preemergence incorporated spray, directed herbicidal spray, and directed insecticidal sprays.

Characteristics of airblast sprayers. In airblast sprayers most spray is formed by conventional hydraulic nozzles. The principle of most high-volume airblast sprayers is not to produce spray drops by air atomization, but rather to provide a large air-jet to transport the spray with enough momentum that the turbulence will not dissipate it until it reaches a certain distance from the spraying machine.

In addition to increased capacity airblast machines provide a turbulent air stream which exposes covered foliage and increases the momentum of medium size drops to improve their deposition by inertial impaction.

Characteristics of aircraft application. Research today indicates that spray discharged along the lateral axis of an airplane will follow the air currents set in motion by its passage through the air, until these forces will not longer support the weight of the spray droplet. Three principal airstreams, which affect spray distribution, are generated

by an airplane in flight. These are the right and left wing-tip vortices and the propeller vortex. These vortices, plus the effect of gravity, determine the path of the spray discharge and the resultant deposit pattern.

Air within the propeller vortex, or slipstream, aside from being driven backward with considerable force, is rotated by the directional effect of the propeller blade causing the column of air driven aft to veer off towards the side until overcome by the adjacent airstreams, and then to parallel the direction of flight. The wing-tip vortices are caused by the flow of air from the high-pressure area beneath the wing diagonally around the wing tips where it spills over into the temporary low-pressure area above the wings.

Essentially the same forces act for high level flight as for low, except that the spray bearing air currents are not so quickly or greatly compressed by contact with the ground.

It is obviously impossible to eliminate propeller and wing tip vortices, and doubtful that these vortices can be sufficiently altered to improve the spray pattern appreciably, although this possibility requires further research before it is completely dropped.

A normal spray pattern from an even array of nozzles along a full wing span boom may be substantially altered by spacing the nozzles, and thus obtain reasonably uniform spray distribution. Nevertheless aerodynamic forces are such that deposit rates vary considerably from foot to foot across and along the swath.

Inversion and lapse temperature conditions have not been found to influence mass median size of deposited drops but do affect drift, especially at high level applications. Since hydraulic nozzles are used on aircraft, drop size distributions have characteristics similar to those of ground equipment.

There has not been much testing of helicopters for spraying and dusting work but tests conducted so far indicate, that other things being equal, the deposit pattern from helicopters is more uniform than from fixed wing aircraft.

FUNDAMENTAL PROBLEMS OF PESTICIDE APPLICATION

The first thing that must be known either accurately or approximately is how much chemical is required and where and how it is to be applied.

It is useful to assume that a pesticide has a definable toxicity.[3] A requisite quantity of available insecticide to cause the death of an insect may then also be defined. The quantity is not constant but may be considered approximately so. In actual practice we apply many times as much insecticide per acre as would be necessary to kill the insects there if it could be distributed directly on the insects and providing that each insect received his "fair share." It is interesting to calculate the number of insects that could theoretically be killed by 1 pound of insecticide if it could be ideally distributed on the insects. For example, USDA entomologists tell us that an "average" boll weevil may weigh approximately 17 mg. and that one part per million of a good phosphate poison is required to kill him. Then:

Approximate weight 17 mg.
Poison required = 1 p.p.m. = approximately $\frac{2}{100{,}000}$ mg.

Then 1 lb. would kill approximately 23,000,000,000 weevils. Assuming a plant population of 23,000 plants per acre, we could kill 1,000 boll weevils per plant on 1,000 acres.

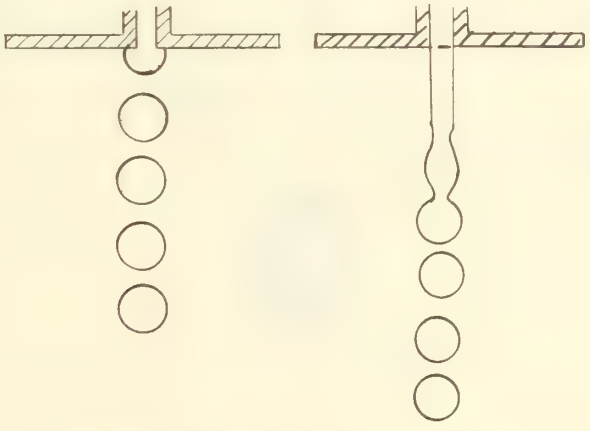
Even much more startling calculations could be made for much smaller insects such as the mosquito and the aphid but such figures only lead to wrong impressions since no field application procedure will ever even approach these figures.

[3] Courshee, R. J. Some Aspects of the Application of Insecticides. Annual Review of Entomology (English) Vol. 5, 1960.

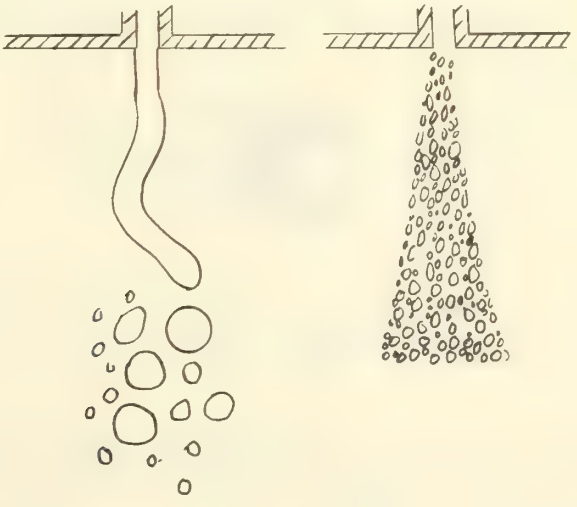
Knowing the amount and required characteristics of the pesticide application, we then can devise a method of forming the particles required, as well as transporting them to and depositing them on the plant. Finally, we are concerned if the particles will adhere to the plant they are deposited upon.

Particle Formation. It is an axiom of engineering that to control the process you must understand the mechanics of the process, and preferably be able to describe it quantitatively. A considerable amount of research has been done on the mechanics of droplet formation yet much remains unknown. It is customary to speak of 3 or 4 different modes of droplet formation. The first is known as dribble where liquid issues very slowly from an orifice and drops are formed one at a time as liquid is supplied to the orifice, such as on a burette or pipette. We understand this process quite well and can predict and control the size of drops which result. As you can easily imagine this process is of little usefulness in practical application of chemicals.

The second mode of droplet formation is known as Rayleigh breakup. In this process the liquid issues from a nozzle in a solid jet at relatively low speed, but at some distance from the nozzle the liquid jet, because of its inherent instability, breaks up to form droplets. Again we understand, can predict and can control the size of droplets formed by this process, but it has not been very useful in field application of chemicals.



The third mode is known as sinuous breakup. At these velocities the liquid jet tends to describe a spiral or sinuous path as it issues from the nozzle and because of aerodynamic forces on the jet it breaks up quite quickly. This process is not well understood but again it is not very useful in pesticide application because of limited discharge capacities.



The fourth mode is known as instant atomization. In this process the jet appears to break up instantly as it issues from the nozzle, due to the very great aerodynamic forces on the liquid. This is the controlling process in most practical spraying applications. Very little is known about the mechanism of this type of atomization, because of the very high speeds of the small particles involved. At this point it would be well to illustrate why it has been difficult to learn much of the mechanism of instant atomization. For example, in a hydraulic nozzle operating under typical conditions, we would have to photograph, preferably in motion picture form, a jet or droplet one-half mm. or less in diameter and travelling 27,500 mm. per/sec., roughly 60 to 70 miles per hour, in order to see what is going on. This is very difficult. This is equivalent to taking a photograph of a golf ball travelling 3,000 miles per hour, assuming equal size images on the film. Photographic equipment developed recently may be capable of this job, but it is quite costly.

Trajectories and Deposition. The trajectory and deposition of pesticide particles are controlled by inertial, gravitational, aerodynamic, thermal, and electrical forces.

When a droplet is formed it is generally given a velocity in some direction. Because of its momentum it will tend to continue in this direction except for acceleration in other directions by various forces, in accordance with Newton's second law of motion. Gravitational attraction will exert a force downward:

$$F_g = \frac{D^3 \rho_p}{6}$$

where D is the particle diameter and ρ is its density.

Because air is ever present aerodynamic drag will exert a force opposite the instantaneous velocity relative to the air on a spherical particle of

$$F_a = -C_d \pi D^2 v^2 \rho_a / 8$$

where ρ_a = density of air, v = velocity of particle, and C_d = drag coefficient, which in turn depends on the Reynolds number $\frac{Dv\rho_g}{\mu}$ where μ is the viscosity of air. For small particles generally $\frac{DvP_g}{\mu}$ is small enough that $C_d = \frac{24\mu}{\rho_a vD}$ is accurate. Then

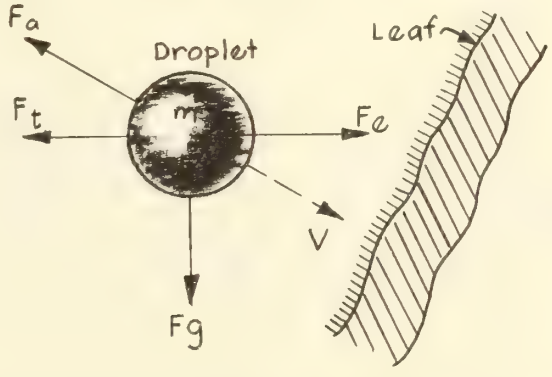
$$F_a = -3\pi\mu vD$$

If the particle is given an electrical charge q and is surrounded by an electrical field with a gradient ∇V, there will be force exerted on the particle of $F_e = q \nabla V$.

The electrical charge resides on the surface of the particle and is a form of surface energy. If the droplet is a conductor, the amount of charge will be limited by the charging system and the subsequent discharge through air ionization. If a nonconductor, the charge will be limited by surface instability, because the surface charge tends to reduce the effective surface tension. In the latter case, the maximum equilibrium surface charge in air of a nonconducting drop is $q = 2.65\ (10^{-9})$, coulombs/cm^2. Thus, the maximum charge on a particle would be given by $q = (2.65 \times 10^{-9})\ \pi D^2$ coulombs so max $F_e = (2.65 \times 10^{-9})\ \pi D^2 \nabla V$ if D is in cm. If the particle is surrounded by air in which there exists a thermal gradient ∇T, the difference in mean molecular velocity on either side of the particle will exert a force on the particle. The force on the particle is given by the expression

$$F_t = \frac{9\pi\mu^2\ D\ R\ x_a}{2pM\left(3x_a + x_p\right)} \nabla T$$

where R = gas constant, M = equivalent molecular weight of the air, p = air pressure, x_a = heat conductivity of the air, and x_p is the heat conductivity of the particle. A summary sketch of the possible forces that may be acting on a charged particle approaching a hot leaf is shown below.



Now you can see that it may be possible that a number of forces will be acting on a particle. These forces depend, among other things upon the size of the particle, the force per unit mass of the particle generally varying with the diameter of the particle. One of the things that is enlightening to study is a comparison of these forces for varying

particle sizes. This can be done by forming ratios of the various forces and see what functions of drop diameter results. However, these particle forces always accelerate the particles to some velocity where aerodynamic drag equals the force. Since aerodynamic drag is always present, another useful comparison is to compare the equilibrium velocity due to the force in question with the equilibrium velocity of settling in still air due to gravity.

The ratio of aerodynamic force to gravitational force is:

$$\frac{F_a}{F_g} = \frac{18\mu v}{(\rho_p - \rho_a)D^2}$$

The ratio of the maximum force due to electrical charges to the gravitational force is given by:

$$\frac{F_e}{F_g} = (1.62 \times 10^{-4}) \frac{\nabla V}{\rho_p D}$$

The ratio of a force due to thermal gradient to force of gravity is given by:

$$\frac{F_t}{F_g} = \frac{18 \mu R x_a \nabla T}{pM\rho(3x_a + x_p)D^2}$$

The ratio of the maximum terminal velocity due to electrical forces to the terminal velocity due to gravity is given by:

$$\frac{V_e}{V_g} = \frac{(2.65 \times 10^{-9}) \nabla V}{D \rho_g}$$

The ratio of maximum velocity due to a thermogradient to maximum velocity due to gravitational attraction is given by:

$$\frac{V_t}{V_g} = \frac{27 \mu^2 R x_a \nabla T}{\rho_p D^2 pM(3x_a + x_p)}$$

With all these forces available and apparently explicit formulas as to how to obtain them, one might wonder what is preventing us from putting sprays and dusts exactly where we want them right now.

Our statement of the problem at the beginning of the paper says that we must apply these chemicals with practical equipment. Practicality involves at least two things--capacity and cost. For instance, if we devise equipment which would yield highly improved deposition efficiencies travelling at only a half mile per hour, it would not be considered practical equipment. Likewise if spraying and dusting equipment with greatly improved deposition efficiency were to have greatly increased initial cost or greatly increased cost of operation, it could not be considered practical. Even with the inexpensive spraying equipment available now, the cost of application, including both the cost of operator time and amortization of equipment, is often equal to, or greater than, the cost of chemicals applied, when such common chemicals as 2,4-D and 2,4,5-T, are used.

Almost everything described so far is concerned with single particles of a particular size. In practice we likely will have a cloud of thousands of particles of a number of sizes. We know for instance that aerodynamic forces interact on particles nearby so that the path of a single particle travelling through the air subjected only to aerodynamic forces is not the same as several particles which are close to one another.

The same is true for electrostatic forces. The action of a great number of particles under the influence of primary forces and interaction of primary forces should be studied, therefore.

In addition, the aerodynamic flow pattern, produced by existing pesticide application equipment, as well as proposed equipment, should be thoroughly studied. This would include aircraft, airblast, and hydraulic spray equipment. The penetration of pesticide sprays into plant foliage should be correlated with various aerodynamic patterns and with the initial energy possessed by the spray. The characteristics of variously shaped force fields and their resulting gradients need thorough investigation to take best advantage of the forces we have available.

We have seen that the forces on a particle depend upon its size. It is also generally believed that the biological effectiveness of a pesticide often depends upon the size of particles into which it is divided. This is usually based on calculations of the theoretical coverage obtained from dividing a certain volume into certain particles of various sizes.

The following illustrates this by showing how 10 gallons of liquid would cover a perfectly flat surface with droplets having a contact angle of 90°:

Drop Diameter Microns[4]	No. of drops per sq. cm.	Mean distance apart in mm.	Percent of one acre covered at contact angle - 90°
10	2,213,500	0.00683	266.0
20	267,000	0.0194	133.0
30	79,100	0.0354	88.7
40	33,350	0.0547	66.5
50	17,080	0.0765	53.2
75	5,600	0.134	35.5
100	2,135	0.217	26.6
150	632	0.398	17.7
200	267	0.610	13.31
300	79.1	1.13	8.87
400	33.3	1.74	6.65
500	17.1	2.24	5.32
750	5.6	4.24	3.55
1000	2.13	6.83	2.66

For plant surfaces these figures would have to be divided by the acres of plant surface per acre of land which may vary from 5 to 20 and multiplied by a spread factor corresponding to actual angle of contact.

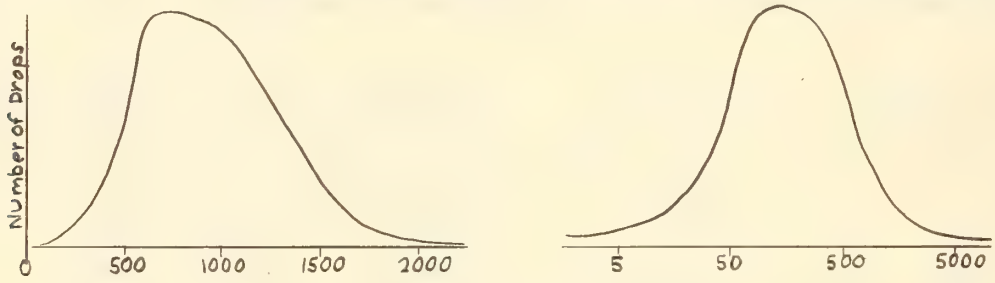
From these figures it is not difficult to appreciate the reasoning in favor of low gallonage--fine droplet spraying. Theoretically, the reduction in coverage resulting from a 50 percent decrease in volume can be offset by reducing the droplet size by only 50 percent. Consequently, it has always been assumed that pest control generally improves as we divide our pesticides finer and finer, although experimental evidence to support this exists for only a few pest-pesticide combinations. It is also reasonable to assume that variable deposits are wasteful.

However, we are certain that, quite often, the finer the droplets the more difficult it is to control their placement. If all applications were made with large spray volumes and with a coarse spray with a low surface tension, we would have less trouble with deposition. But because of dusting, low-volume spraying, and perhaps sprays of higher surface tension, deposition is difficult.

[4]One micron equals approximately 1/25000 inch, 25 microns equals approximately 1/1000 inch.

Thus, we need research to obtain for each pest-pesticide qualitative or quantitative curves showing the relationship between biological reaction and particle size. While we would expect the biological action to improve as the particles become finer, perhaps there would be a point below which the biological reaction would not greatly improve. This we would then consider the optimum particle diameter for controlling this particular pest with this particular pesticide. It would be the largest particle which would provide reasonable control. We would then aim to divide our pesticide into particles all of which were approximately this size.

Another problem would then present itself. When we try to produce a large number of small particles of a particular size, we do not get a single size particle but a wide range of particle sizes. Generally we find that the particle size distribution we get is some type of logarithmic normal distribution. This distribution when plotted on conventional scales looks like illustration (left) below. When plotted on logarithmic scales, it will appear as illustrated (right) below. This distribution has the characteristic that a great many small droplets constitute a small percentage of the total volume and, conversely, a relatively small number of droplets make up a large percentage of the total volume of spray. From our discussion of the effect of various forces on droplets of different sizes we can see that this large number of small droplets is undesirable.



It is reasonable to ask why we get such a distribution of particle sizes. Apparently it is due to the fact that the particle size depends upon a number of variables, each of which in turn is subject to random variation.

What can be done to minimize the geometric standard deviation of this logarithmic normal distribution, or what can be done to reduce the tendency towards a log normal distribution itself? To reduce the variability of a drop size distribution caused by some drop formation process we could do two things. We could minimize the variability of each of the components variables which affect the process, and we could reduce the number of variables. Reducing the number of variables which affect the process should also tend to make the drop size distribution less nearly log normal.

Measurement of Mass and Particle Size Distribution. The problem of measuring chemical distribution is one of our most critical because all other research hinges upon it. This will include highspeed measurement of particle size distribution, quantitative measurement of chemical distribution on the soil, and quantitative mass distribution of chemicals on plant surfaces. Until we have these methods available, all other research on pesticide application equipment will have a corresponding lack of precision.

In addition, the effect of natural meteorological conditions upon pesticide application requires further research. This includes study of the velocity, scale and distribution of atmospheric turbulence, and the effect of humidity on electrostatically charged particles.

Particle adhesion. Despite its great importance to pesticide application we have almost no basic information on the adhesion of particles for various surfaces. Most of the basic work which has been done so far on particle adhesion has been done by the people working on air filters. Research is badly needed on physical mechanisms in the retention of materials on plant surfaces.

Characteristics and use of special materials. Chemicals in granular and fumigant form are increasing widely in use but little basic research has been done on the mechanics of application of these materials. More accurate metering equipment is needed for granular pesticides. Methods should be developed to determine whether granular material will break up during a particular metering or application process.

We also need more accurate metering and application equipment for fumigant chemicals than are available at the present time. The effect of soil nonhomogeneity upon pesticide action throughout the soil needs considerably more research, as well as methods of sealing soil pesticides in the soil.

FUTURE PROBLEMS

Basic Research. Much progress has been made in pesticide application by following the more obvious ways and means of applying pesticides. No apologies should be made for this procedure since the applicability of the results and the benefit to agriculture to date have undoubtedly been highest with this procedure. However, as with other progress, the easier gains have been made and there is urgent need for expanded basic engineering research on the physical forces (gravitational, inertial, thermal, electrical, and viscous) which control the deposition of pesticides. The need then is for basic research to define the fundamental physics of aerosol behavior.

Applied Research. Continuous research will be necessary to develop machines and techniques for more accurately directing and uniformly applying all types of pesticides, liquid, dust, and granular, to all important crops.

As entomologists define insect pathogens which are harmful to insects but not to higher animals and plants, equipment will be required to properly dispense and distribute these materials. For example, research reports indicate that virus of the tent caterpillar can be concentrated so that a solution of material weighing 1 pound will furnish control when spread evenly over 50 acres of trees. Such an application problem challenges the imagination.

Aerial applications, including those by helicopters, will continue to advance with increasing knowledge of the requirements.

In conclusion, we wish to restate our firm conviction that greatest progress will come with a well coordinated and integrated effort, including those of the engineer and the biological scientist.

SELECTED REFERENCES

(1) Brazee, Ross D., and Wesley F. Buchele. Electrostatic Precipitation of Agricultural Dusts, An Outline of Research and Literature. U. S. Agr. Res. Service ARS-42-29, (July 1959), (Contains a summary of publications related to electrostatic precipitation of dusts)

(2) DeJuhasz, K. J. Spray Literature Abstracts. Am. Soc. Mech. Engineers (1959). (Abstracts of about 1300 literature items on sprays, atomization, aerosol mechanics, and nozzles. Includes experimental and measuring methods and instruments, theories, and application).

(3) Fuks, N. A. The Mechanics of Aerosols. Translated by M.S.P.E. Lachowicz. U. S. Dept. of Commerce, Office of Technical Services Translation 59-21069 (1959) (A comprehensive monograph of aerosol mechanics, presenting work from 580 references. Emphasis on smaller aerosols, usually $D \ll 100$ microns.)

(4) Giffen, E. and A. Murazew. The Atomization of Liquid Fuels. Chapman and Hall, London (1953). (A review of research on spray formation.)

(5) Herdan, Gustav. Small Particle Statistics. Elsevier Press, Amsterdam. 1953. (Entire monograph deals with statistics involved in sampling and describing size distribution of dusts and sprays.)

(6) Marshall, W. R., Jr. Atomization and Spray Drying. Chem. Engin. Prog. Monograph Series No. 2, Vol. 50, A.I.CH.E. (1954) (A review of research on spray formation, including air atomizers and spinning discs, and review of drop evaporization.)

(7) Ranz, William E. On Sprays and Spraying. Engin. Res. Bul. B-65, Pennsylvania State University (1956) (A review of theory and experiments on sprays. Includes droplet stability and inertial impaction.)

(8) Whitby, K. T. Bibliography of Particle Size Determination. Univ. of Minn. M. E. Dept., Milling Engin. Res., Minneapolis (1949) (Outlines 27 methods of particle size determination, and contains a bibliography of 450 titles on this subject.)

(9) Basic Considerations in the Combustion of Hydrocarbon Fuels in Air. National Advisory Committee for Aeronautics Report 1300. Washington (1959) (Chapter 1 provides a concise review of mechanics of spray formation.)

(10) Handbook on Aerosols. Atomic Energy Commission, Washington (1950). (Compilation of reports on various phases of research on aerosols with D<<100 microns.)

USE OF INSECTICIDES TO PROTECT STORED GRAINS, FRUITS, AND VEGETABLES

L. S. Henderson[1]

The discussion of this topic involves a slightly different approach than was followed in the other parts of the symposium. We are dealing here with chemicals in relation to harvested crops and processed plant or grain products rather than with the application of chemicals to growing plants. A definition of the boundaries of the subject matter to be covered will be helpful. We will deal with stored grains as a raw agricultural commodity and also with the flour and various cereal products derived from grains. There are not many insect problems with harvested fresh fruits and vegetables, but the problems may be serious with dried fruits and dehydrated vegetables. Pet foods and mixed animal feeds are included, but a number of other commodities dealt with in research on stored-product insects will be omitted even though there are residue problems involved.

It is apparent that many stored products are about to be consumed as food or animal feed. Contamination of these products by insects or pesticides is critical. There is no opportunity, as in the treatment of field crops, for the residue to become depleted through weathering or aging. Many of the products are already processed and in those cases the residue cannot be removed by processing. The public is becoming increasingly demanding that food be pure, clean, and wholesome, and free of insects as well as pesticide residues. The requirements of the Food, Drug, and Cosmetic Act place restrictions on contamination of foods with insects or pesticides. Sometimes there is a rather delicate balance where pesticides must be used to prevent insect infestations in foods, and contamination with the pesticide must be avoided.

[1] Entomologist, Market Quality Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

Dr. Byerly brought out a point in his talk that is of great importance in relation to stored-product insect control. He stated that present population growth indicates the possibility of food shortages within 20 years. Therefore it seems imperative to me that we must learn to preserve every pound of food we can produce. The time to do that is now rather than wait for an emergency to develop. Even today, with current high costs, it is foolish to invest in all that is required to produce a crop and then let insects destroy, damage, or contaminate it.

The problems we are discussing are not limited to this country. In December 1959 there was an excellent release from the European and Mediterranean Plant Protection Organization. It was a "Report of the Working Party on Crop Production Pesticides" and is entitled "The Need for Chemical Control and the Steps Taken to Safeguard Public Health." I recommend it to you for reading and quote one sentence that is pertinent to this discussion: "Up till now attention has been directed mainly to agricultural crops, since pesticides have chiefly been used in the field; but there is a growing awareness of the serious losses of stored foodstuffs due to infestation, and pesticidal treatment is becoming more frequent."

A wide variety of chemicals is used against stored-product insects. They are applied as fumigants, aerosols, space contact sprays, residual sprays, or dusts. They may be applied near or directly onto commodities and careful consideration must be given to avoiding harmful residues. In stored-product insect work all components of the formulation must be considered in this respect, not just the active ingredient. Hazardous carriers, solvents, and emulsifiers must be avoided. Care must also be taken to avoid any adverse effect of the formulation on odor, flavor, grade, or quality of the product, as well as on the structure and its furnishings, machinery, and processing or handling equipment.

Pesticides serve numerous functions in protecting stored products against insects. They are applied in warehouses to kill in the structure itself the insects that would otherwise infest bulk, bagged, or packaged commodities. They are applied directly to bulk grain as fumigants or protectants. They are applied in transportation facilities to protect raw or processed, bulk or packaged commodities during transit. Raw materials held at processing plants for a long time must be protected with fumigants or sprays. Pesticides are applied in the plants to prevent infestations that would otherwise invade the product. The finished product is sometimes fumigated at the time of packaging to insure freedom from infestation. A wide variety of conditions and products is encountered in such establishments as flour mills, bakeries, corn mills, canneries, dehydrating plants, candy plants, feed mills, pickle works, and wineries. The variety and the number of conditions increase as the degree of processing and preparation before sale becomes greater and as new kinds of products continue to appear on the market. Finally, chemicals are used on the packages themselves to prevent insect infestation between the time the product leaves the plant and when it is bought by the consumer.

You will notice that in the preceding discussion we have emphasized the protection of products. We must prevent insect infestations in foods and their raw materials rather than kill the insects. After the infestation becomes established it is too late because the damage has already been done. The insects have already caused destruction, loss, and contamination. Kill out the infestation and the dead insects are still there. The concept of prevention evolved slowly, especially in relation to the handling of some raw materials, but it is now rapidly gaining wide acceptance.

Having considered the stored-product field on a broad basis, let us now look at some specific areas to see where we stand.

GRAIN PROTECTANTS

We will start out with grain protectants because they represent one of our uses of pesticides on a raw agricultural commodity soon after production of the crop. A grain protectant is a treatment applied throughout the mass of bulk grain as it goes into storage bins, to prevent insect infestation during the storage period.

We have studied protectants for several years and have learned a great deal about them. Some appear to function through repellency, some through toxicity, and others through a combination of both actions. Some materials or some levels of application will protect only uninfested grain, whereas others will also kill off existing infestations. There is a relation between the level of residue present in the grain and performance of the protectant. The rate at which the residue disappears governs the length of time it protects the grain. Such factors as grain moisture, grain temperature, and chemical nature of the protectant have an inter-related effect on the performance and on the rate at which the residue disappears.

Protectant studies have been made on several grains such as wheat, corn, grain sorghum, and rough rice. They have been extended to farmers stock peanuts and were tentatively explored even in such products as stocks of dried raisins during the storage period before final cleaning and processing. The materials tested have included ryania, lindane, methoxychlor, synergized pyrethrum, and malathion. Part of the studies has included determining the fate of residues during the milling of treated wheat. Materials such as ryania and lindane have been dropped from further testing because of toxicological and residue factors. Tolerances have been established under the Miller Amendment to permit the use of malathion or pyrethrum plus piperonyl butoxide as grain protectants.

We are continuing our search for materials that more closely approach our concept of the ideal protectant. Just what properties do we need in this ideal treatment? It should be long-lasting with a low residue hazard. It must be economical and easy to apply. It should not be sensitive to high temperature or high grain moisture, and must be effective against a wide range of insect species. It should destroy any existing infestation as well as protect against reinfestation, because grain is sometimes infested when it is harvested. It should not affect germination of grain and must not make the grain undesirable or disagreeable to handle in commercial channels. It must not have any adverse effect on grade, odor, flavor, or baking qualities.

CHEMICAL ANALYSIS

From the very beginning of the research on protectants it was evident that residue analyses were absolutely necessary as an integral part of the research program. There are many other phases of stored-product insect work where chemistry and residue analyses are important, but a discussion in relation to protectants will serve as an example to illustrate the advances that have been made in the use of chemistry.

Chemical analyses are necessary to study and evaluate the rate of disappearance and fate of residues, as well as to correlate the level of residue with the degree of biological performance. Residue analyses are important not only as a research tool but also for obtaining data to be evaluated in the establishment of tolerances under the Miller and Food Additive Amendments. These analyses also indicate to us how much unwanted or incidental contamination may result from a particular procedure in pesticide application.

It is evident that much of the research on stored-product insects requires chemistry as a supplement and that as soon as we start to use pesticides we are immediately confronted with problems of potential residues and contamination. Therefore, it has become almost a standard procedure to set up parallel biological and chemical tests in many phases of our research program. In some cases there is even a preliminary conference with Food and Drug Administration officials to determine whether the planned chemical analyses will provide them the kind of data required to consider the establishment of tolerances. Although we receive much analytical assistance from the pesticide industry, it is impossible for them to provide the number and kind of analyses needed, to time them according to our requirements, or to provide close liaison with our research program. Therefore, the Stored-Product Insects Branch operates its own chemical analytical laboratory.

The extensive use of chemistry makes the experimental program more complicated and more costly. The development of simpler and more rapid analytical procedures would be highly beneficial in research. There is also a need for simple, rapid methods that can be used in trade channels--methods that do not require expensive, elaborate equipment, and that can be handled with little or no laboratory facilities and by people who are not necessarily trained chemists. In some cases even purely qualitative rather than quantitative results would be of great assistance to a grain buyer, an elevator operator, a miller, a feed manufacturer, or a livestock feeder. Such tests would at least tell whether residues were present in sufficient amount to require further and more detailed checking. They might also indicate whether it would be possible to apply a fumigant or protectant without exceeding an established residue tolerance, whether it would be safe to use a commodity for some specific purpose, or whether some unapproved treatment had been applied in ignorance, in error, or even by intent.

FUMIGATION

Tremendous advances have been made in the fumigation of stored products during the last few years. A step that can almost be considered as a break-through is the use of gas concentration rather than applied dosage as a basis for conducting not only fumigation research but also applied fumigations. The analysis of fumigant gas concentrations in air is nothing new but until fairly recently was a complicated, time-consuming operation requiring a well-equipped chemical laboratory. The application of the thermal-conductivity principle to the analysis of some of the common fumigants and the development of portable equipment that can be used in the field either for research or practical fumigations has been the key to this progress. The development of relatively inexpensive gas chromatography equipment has also aided in fumigation research.

The evaluation of fumigation results in relation to applied dosage can give at best only empirical results. Factors such as sorption and leakage affect the actual gas concentration, which governs the results obtained. By being able to determine gas concentrations we can also study distribution of a fumigant, channeling of gases, and other conditions that may affect the results of a fumigation. Furthermore, by keeping track of gas concentrations during a commercial fumigation, it is often possible to take immediate corrective steps to insure a satisfactory job, rather than wait until the operation is completed to discover a failure and then have to repeat the whole job.

The aeration of grain to maintain satisfactory physical condition has gained wide acceptance in the last few years. A fortunate coincidence showed that the same engineering principles of volume and rate of airflow as well as the arrangement of fans and ducts could be used to force a fumigant gas through bins of bulk grain. Experiments showed that adequate and uniform distribution of gas could be obtained, even in large masses of grain in flat storage or silo-type elevators where it had been necessary previously to turn the grain to fumigate it. A portable aeration system has even been developed to fumigate bulk grain in railroad cars.

Light-weight, gas-tight plastic films and coated fabrics are now available and have permitted several innovations in fumigation procedures. Tarpaulins made of such materials can be placed over stacks of commodities to provide a fumigation enclosure under many circumstances where a fumigation would otherwise be impossible. A portion of the contents of a warehouse can be fumigated without treating the entire structure or other contents. The tarpaulins are sometimes left in place to provide an extended fumigation period, plus a physical barrier against reinfestation after the gas has all dissipated. In the khapra beetle eradication program entire structures have been wrapped in tarpaulins and fumigated. One such operation undoubtedly will be remembered a long time in fumigation history. A large establishment was covered and fumigated as a single unit, the job requiring 9-1/2 acres of tarpaulins and 12 tons of fumigant.

When the time came to establish tolerances for fumigants under the Miller Amendment, it was surprising to discover how little residue data were available. Industry and

government cooperated in an extensive program to obtain the necessary data. All the common fumigants are now covered by tolerances or exemptions for use on all the major grains.

The establishment of tolerances has brought about some new problems. Repeated fumigation of grain may be required during long-term storage and may build up excessive residues from continued use of certain materials. Alternate fumigants have had to be used in some storage programs. Those in the grain industry are also faced with the problem of not knowing the previous treatment history of grain they receive. Until they have chemical analyses made, they do not know how much residue may be present or whether they dare to fumigate again without exceeding the tolerance.

We have learned that some fumigants do not air out of grain as readily as we formerly thought they did. There has been some indication that an excessive amount of ethylene dibromide in oats fed to laying hens may have an adverse effect on egg production. The Department issued a press release to warn the poultry industry of this potential hazard and to caution that poultry feed should be aerated thoroughly after fumigation. We are having research conducted under contract to learn more about the problem.

Large numbers of flat storage facilities for grain are being built. There is wide variation in the design of the aeration systems and some are poorly engineered. We must learn how best to use these systems for forced-distribution fumigation. Uneven distribution of fumigant will produce poor insect kill in areas of low gas concentration and excessive residues in areas of high concentration.

Another problem facing us is the need for tolerances or exemptions under the Food Additive Amendment to cover the fumigation of processed foods and animal feeds. The only fumigant tolerance established is 15 p.p.m. of ethyl formate for the fumigation of cashew nuts. Several extensions have been granted, only two of which carry tolerance specifications. One is for 25 p.p.m. of hydrogen cyanide as a fumigant. The other is for 50 p.p.m. of inorganic bromides in processed foods resulting from methyl bromide fumigation. An extension for ethylene oxide specifies a dosage rate of 0.4 ml. per pound of dried fruit. There are extensions for carbon tetrachloride, chloropicrin, ethylene dibromide, and ethylene dichloride as fumigants for food plant use. Presumably this covers their use on processed foods in a food plant but a strict interpretation would preclude their use in a warehouse. An extension granted for propylene oxide permits its use as a fumigant in foods. As stated in the extensions, none of the materials except hydrogen cyanide could be used on mixed or processed animal feeds. It appears obvious that a large amount of residue data will have to be obtained to establish all the final tolerances or exemptions needed.

We also need to do a great deal of basic research on background factors related to fumigation. It is surprising to find how little is known about the behavior and distribution of fumigant gases in mixtures or under vacuum, the fundamental aspects of penetration and sorption, and the performance of fumigants at lower temperatures. There should also be a search for new fumigants with improved properties. Our Branch is now in the process of initiating some limited work of this nature.

FOOD PROCESSING PLANTS

One of the most urgent problem areas is that relating to the use of pesticides in food processing plants. The food and pest control industries, not to mention many in government circles, are thoroughly confused as to what pesticides can be used safely in food plants, or where and how they can be applied without coming into conflict with the Food Additive Amendment. The industry is concerned because on the one hand they must use pesticides to prevent the insect infestations that would be in violation of the Food, Drug, and Cosmetic Act, and on the other hand they greatly fear that some unintended or unknowing misuse of a pesticide may bring about a Food and Drug action. Where the reputation and good will of a brand name is concerned, a Food and Drug citation causes an unfavorable and costly public reaction.

Not a single tolerance has been established under the Food Additive Amendment for any standard insecticide or fumigant to be used in the entire processed food and animal feed field. There are extensions granted for the use of pyrethrum plus piperonyl butoxide and a few of the common fumigants in food plants. Through prior approval pyrethrum and piperonyl butoxide can be used as a treatment for multiwall paper bags.

The reason for the state of confusion and the lack of tolerances is that essentially no scientific research has been conducted on the use of pesticides in food processing plants. Background knowledge about the properties and other uses of materials has been drawn upon as the basis for estimating by inference what might happen if they were used in food plants. By trial and error general observations have been made as to which materials are effective in practical use.

There is an urgent need to determine which pesticides can be used safely as well as effectively under the numerous and diverse conditions that exist in food plants. Attention must also be given to where and how the materials are applied within food plants. There must be residue analyses on the product from the normal flow line to determine whether or not contamination results from various uses of different pesticides. Dr. Byerly has referred to the difficulty of insuring that we can meet zero tolerances and stated that it is not possible to prove absence of residue but only presence. Therefore, the research of which I speak will be time-consuming and complicated but it appears evident that eventually the work must be done.

In the meantime, those who use pesticides and those who make recommendations for their use in food plants have only the most general guides for procedure. About the best we can do is to say that pesticides shall be used so food will not be contaminated. In the absence of residue data there are many borderline circumstances where nobody is quite sure whether or not contamination will result. There are differences of opinion among regulatory officials as well as among researchers as to what constitutes a hazardous use. Strong personnel opinions are sometimes based on prejudice rather than fact. Until more facts are known we need some sound thinking and a lot of commonsense to provide guidelines to the industries involved as well as to regulatory agencies. This would also serve to settle much of the apprehension and anxiety that now exist.

TRANSPORTATION FACILITIES

Another urgent problem area for the food, grain, and feed industries is the infestation hazard during transit in railway cars, trucks, and ships. Commodities that become infested in transit may be refused by the customer or subject to action by the Food and Drug Administration. Either procedure is costly and of considerable importance to the shipper or processor.

Better ways must be found to eliminate infestations in transportation facilities and to protect commodities against insect invasion during transit. The procedures developed must not cause residues or have other adverse effects on the commodity.

The problem has become so acute in rail transportation that we are setting up a project to get some exploratory work started on it. There has been a considerable amount of discussion recently about the insect problem in ships, but we are not in a position to start any work along these lines now. This situation should not be permitted to continue indefinitely. When our grain and other products are infested upon arrival at foreign ports, it casts a reflection on the quality of our goods and this is detrimental to international trade. The recipients may not realize, or care, that the products were insect-free when shipped and that the infestation occurred in transit. Infested ships also increase the hazard of introducing harmful pests into this and other countries. As an example, the port inspectors are finding an increasing number of ships and cargoes infested with the khapra beetle right at a time when we have exerted a great deal of effort toward eliminating the known infestations in our country.

There are international complications in dealing with some phases of ship infestations that bring about difficult situations to handle. We have discussed this with an official from the Rome office of the Food and Agriculture Organization. It is possible that a cooperative international approach can be arranged by setting up a liaison through that office.

PACKAGING

A food processor loses the benefit of all the effort and expense devoted to producing and packaging an insect-free product if the package is invaded after it leaves the plant and the product is infested when it reaches the consumer. Adequate protective packaging is costly, sometimes out of economic balance with the value of the product inside the package.

We have conducted extensive research for the last several years, with the objective of developing more effective, economical, insect-resistant packaging for foods and other susceptible products. A great deal has been learned about the problem and some significant advances have been made. The research is divided into three phases--package construction and closures, the materials used as package components, and treatment that may be applied to impart insect resistance.

Industry has been extremely interested in this research and we have derived tremendous benefits from their cooperation and participation in the program. In a single large-scale test we have had as many as eight or nine cooperators from the insecticide, paper, container, and food industries. Each cooperator contributes some material or service, and by pooling these resources we can accomplish much more than by individual effort.

We have learned that good package construction and closures are highly important in preventing insect invasion. In one test to evaluate several different types of closures, the preliminary results were so striking that a cooperator spent $100,000 for a new machine to provide a different kind of wrap for his product, even before the experiment was completed.

A number of kinds of plastic films and other materials used in package construction have been evaluated for their ability to resist insect penetration. The information obtained is highly beneficial in selecting the best components for packages.

Simple laboratory tests have been devised to evaluate the effectiveness of chemicals as potential treatments to increase the insect resistance of packages. Promising materials are further tested under simulated warehouse storage conditions in the presence of heavy infestations of about 15 species of stored-product insects. Some experimental treatments have given protection against invasion and penetration for 12 to 15 months.

In using chemical treatments the potential migration of the chemical through the package and into the product must be investigated. A simple test method has been devised to use in the search for effective barrier films and other means for precluding such migration. The method now includes chemical analysis to detect the migration of the material used for treatment. It is anticipated that radioactive tracer techniques would facilitate this work and greatly speed up the detection of chemicals in following their migration. We are in the process of installing a tracer laboratory and this is one of the uses to which it will be put.

We are interested in the possibility of using repellents rather than toxicants for package treatments. An efficient repellent could be a compound with low mammalian toxicity, thus reducing the residue hazard. Furthermore, it would be more desirable to keep the insects away from food packages than to kill them after they crawl over the containers. This would avoid the presence even of dead insects. We have evaluated over 800 compounds as potential repellents and several promising ones are in the second phase of testing.

WAYS TO SOLVE STORED-PRODUCT INSECT PROBLEMS

The discussion thus far has presented some of the insect problems associated with stored products and described our progress in solving them. It is appropriate that we should now give some attention to further research needed. It is only natural that the current emphasis on pesticide residues should influence the trend of planning for future research. We must find ways to reduce or eliminate residue hazards.

The popular and timely thing to do now is talk about the use of insect parasites as a substitute for pesticides. This approach may be practical and effective for controlling insects in the field, but it is not for those attacking stored products. A number of parasites of stored-product insects are known. They are often abundant where a commodity is heavily infested. The difficulty is that there is always a lag between population increase of the pest species and the time the parasite brings the pest under control. By that time there are too many of the pest insects present to be tolerated in a stored product. Contamination has already occurred and much feeding damage has resulted. Furthermore, the presence of the parasites in most instances is just as objectionable as the host species. There are very few people who would relish a bowl of breakfast cereal or a slice of bread with insects in it, even though they were informed this was a beneficial parasite used to prevent contamination of their food with stored-product insects. Those who wish to give impetus to the use of parasites against stored-product insects should explore with the Food and Drug Administration the possibility of establishing tolerances for parasites in food.

There have been exploratory trials in which fungi that attack stored-product insects have been used. These have not been successful, probably because moisture levels under storage conditions are so low that fungi do not thrive. There may be possibilities for using other insect pathogens such as bacteria, viruses, or protozoa. We are initiating some work to explore the possible use of Bacillus thuringiensis against pests in storage warehouses. A possible limiting factor in the use of pathogens is the specificity of many of the organisms. In stored-product entomology a problem is seldom limited to one or even a few species. Measures used must generally be effective against a large number of diverse species. There are exceptions where the use of pathogens could be appropriate.

Attention should be given to physical or nonchemical means that could be used against stored-product insects. A number of forms of radiant energy are effective. These include high-frequency sound or electrical waves, gamma rays, cathode rays, and others. The difficulty has been to develop the radiations in sufficient quantity or intensity, to produce them economically, or to handle them efficiently and safely enough to treat large quantities of products as rapidly as must be done under commercial conditions. Technological developments are gradually making improvements that may eventually enable practical applications to be worked out.

Greater attention should be devoted to such factors as sanitation and thorough cleaning, handling methods for raw and finished products, and other operational procedures in relation to insect problems. Even the manipulation of environmental factors may be utilized as a corrective measure. The ventilation of an area or the aeration of a commodity may be helpful. Controlling the temperature, relative humidity, or the moisture content of a product may eliminate some insect problems. All of this is especially pertinent in the processing industries, where the maintenance and the nature of construction of buildings, machinery, and equipment are also highly important.

It will be noted that the preceding emphasizes prevention rather than control. A very few firms have already learned that they can reduce to a minimum the needs for pesticide applications by proper attention to these matters. There is an urgent need to apply technical entomological knowledge to the subject. We feel this is just as truly a research as some other areas of investigation, and that it could be highly productive of valuable results. It has received little attention and not much practical advantage has been taken of what is already known. One of the needs is to obtain figures that will prove to management that money spent along the right lines will bring about savings in the long run.

Several lines of research are needed in connection with the use of chemicals. We have discussed the work being done on repellents for package treatments. We are now beginning to look also for repellents that might be applied to grain for protection against insect infestation during storage. Other potential uses for an effective repellent would be in warehouses, transportation facilities, or food plants. The search for repellents is challenging because it may be possible to find effective materials in groups of chemicals that lack mammalian toxicity. This would be another step toward avoiding pesticide residues.

The search would be continued for effective pesticides with low toxicity to humans. At the same time we should be alert to take advantage of unique physical and chemical properties of compounds. There are intriguing possibilities for the use of vapor toxicity or a short residual life in the stored-product insect field. Other compounds that may be of interest are those that interfere with or interrupt normal insect growth, development, or reproduction.

We believe it would be profitable to devote some more careful study to formulations and methods of application for the pesticides we are now using. If improved formulations were devised to produce increased effectiveness, less pesticide would be required to do the job and residue hazards would be reduced. Better application methods would reduce the possibilities of incidental contamination and increase the safety to operators.

Finally, and perhaps most important of all, a great deal of basic research on the stored-product insects themselves is much needed. It is surprising to find how little is known about many of the species. Most of the information we have about the more common species was obtained under controlled laboratory conditions of constant temperature and humidity, often in the range optimum for the insects. There is little reason to assume that the biological functions of growth, development, reproduction, and behavior will be the same under these conditions as in the natural habitat. We should conduct ecological, life history, and behavior studies under the actual environmental conditions of the grain bin, elevator, warehouse, or food plant. Only then will we begin to learn how these insects really live. Also is needed some basic research on the reactions of these insects, their responses to various stimuli, competition and interaction between species, their migration or movement from place to place, sources of infestation, the ways and reasons infestations originate, and the relationships between infestation and different commodities or different kinds of food supplies. As we learn more about the insects we will be able to devise better ways of protecting our food and other commodities against their ravages.

INDUSTRIAL CHEMICAL CONTAMINATION OF LIVESTOCK FEEDS

Aubrey M. Lee[1]

The problems on industrial chemical contamination of livestock feeds will be discussed in this paper. Excluded are the biological agents in the nature of viruses, bacteria, rickettsiae, parasites, and mycotic agents, which we know may contaminate livestock feed and about which we need so much more scientific research information. Also excluded are discussions on the contaminations of livestock feeds with chemicals that are poisonous because of biological and chemical activities within the soil, plant, and animal. These numerous exclusions are as important and, in most cases, more important than industrial chemical contamination of livestock feeds as far as the health of farm animals are concerned. They are too numerous to mention but some include several trace minerals, especially selenium and molybdenum, and the currently important

[1]Head, Noninfectious Diseases Section, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture.

nitrate-nitrite-methemoglobinemia complex. This toxicity for cattle, sheep, and sometimes swine, from the presence of excessive nitrates in livestock feeds is being diagnosed, reported, and published with increased frequency by several State agricultural experiment stations. It may become a concern of major importance to regulatory officials and is deserving of all-out research efforts.

The pesticides and feed additives which are probably, in most instances, neither industrial nor nonindustrial contaminants except by accident, mistake, error, or failure to use as directed or labeled, will be discussed by others in this symposium. Frequent localized losses of animals from errors are of importance to the individual owner and the veterinarian. They are often reported in veterinary journals. Investigations of such losses help contribute to the knowledge of veterinary toxicology of the respective chemicals, but are expensive to the farmer. As an example, the killing of four milk cows and the sickening of five others from the feed of a local reliable feed company was recently reported in the Journal of the American Veterinary Medical Association. The parties concerned agreed that the chemical and pathologic findings confirmed poisoning from the urea in the feed. Such types of problems will not be included in this paper, nor will industrial contaminants of livestock feeds, such as ethylene dibromide and other fumigants that are being used on stored grains for protection from destructive agents.

We need scientific research on the problems of tissue and milk residues; the effect of the chemicals in feed and the metabolites they produce on the offspring when ingested during stages of pregnancy and their importance as producers of duress in triggering disease production by known and unknown infectious agents.

To determine a specific industrial chemical contamination of livestock feeds is difficult. There are many reasons for this. The toxicity, which requires a long period of time to produce, is often a chronic one. The particular batch of feed or part of pasture has been exhausted by the time its toxicity is known. Only one species of livestock may be affected. Entirely different symptoms and lesions may be produced in different species of animals. The state of nutrition of the livestock may be an important factor in the severity of the toxicity. Some poisonings are characterized by the production of certain enzymatic changes, which give them characteristics of some nutritional deficiency or imbalance. A very economical, productive, or practical process or method of production may be involved. There are often fears and warnings about the damage of a particular crop, a practical economic process, or a popular product. Toxic feed from one area where it is grown or stored may be used by a particular livestock feed processing plant, and nontoxic feed from another area may be currently processed at the time the toxicity in livestock is exhibited. The grease or lubricant used on the processing machinery may be changed. There are more reasons and examples of each which could be cited.

At this time, we know of no proved or factual industrial contamination of livestock feed that is causing symptoms and lesions in livestock or poultry over any wide area or in numerous communities.

The industrial chemical contaminants of livestock feeds of the past that caused sickness and death of animals over many and wide areas of the United States, include lead, arsenic, fluorine, nitrogen trichloride, free gossypol of cottonseed meal, trichloroethylene-extracted soybean meal, and chlorinated naphthalenes. The air pollutants, which have been the cause of most known damage to livestock through recognized production of symptoms, have been lead, arsenic, and fluorine (Phillips, 1956). This was not because they were present in the air in amounts to cause trouble in breathing, but because they accumulated as deposits on forage and vegetation that are later consumed by animals. However, in the last 20 years industry has made great progress in the removal of arsenic and lead from industrial stacks as a source of contamination of livestock forage by air pollution.

Some coals contain as much as 54 p.p.m. of lead as an impurity. In the vicinity of a coke oven the herbage was found to contain 25 to 46 p.p.m. of lead, which was sufficient to cause poisoning of cattle and sheep (Phillips, 1956). Dusts and sprays from smelters, where ores are smelted to obtain metals are other sources of a contemination of livestock feeds.

The Animal Disease and Parasite Research Division currently has a contract for research on one small phase of lead poisoning. There are extreme variances in the literature regarding susceptibility, tissue residues, chronic poisoning, and the lead levels of industrial contamination of livestock feeds. The relationship of levels of lead, which livestock ingest, to the amount required to produce residues in meat and milk and symptoms in the animals is an area of needed research. Binns and Beeson (1959) report that there may be effects from a nonlethal dose of lead at a certain period of gestation.

The diagnosis of lead poisoning with certainty is difficult even with chemical analyses. It is generally considered that for evidence of diagnosis of lead poisoning, findings in the undried tissues should be 40 p.p.m. in the kidney cortex or 20 p.p.m. in the liver. Lead is retained by the organs for a long time and abnormally high blood levels have been seen 6 months after ingestion of a single sub-lethal dose (Allcroft, 1951). However, deaths occurred from unquestioned lead poisoning in calves in which the liver contained from 9.5 to 182 p.p.m. and the kidneys from 0.5 to 300 p.p.m. (White, et al., 1948). Cows have experimentally tolerated 2 gms. each daily for 2 years without apparent harm (Allcroft, 1951). Calves up to 4 months of age are usually killed by ingestion in 1 day of 200 to 400 mg./kg. of the acetate, oxide, or carbonate. With cattle and sheep, it is extremely unlikely that cumulative poisonings occur naturally to produce the chronic poisoning, possibly due to the low 1 to 2 percent absorption of the lead ingested (Blaxter, 1950). We should expect little actual lead poisoning of cattle and sheep from industrial contamination of livestock feeds from air pollution or from the greases and lubricants that get into feeds, particularly pellets.

Arsenic also contaminated livestock feeds by air pollution from various industrial processes, such as smelter fumes or coal containing arsenic as an impurity. Grain dried by direct contact with flue gases has been reported to have an arsenic content of 0.2 p.p.m., when the coke used to produce the flue gases contained 80 p.p.m. of arsenic (Phillips, 1956). Chronic poisoning occurred in animals grazing on land over 6 miles from precipitated fumes from smelters and blast furnaces using ores containing arsenic. Toxicity from spray and dust contaminations of livestock feeds have been reported. Many factors involved make it impossible to state the toxic dose to any one species. The average lethal dose according to Garner (1957) for sodium arsenite for the cow is 1 to 4 gms. and 0.2 to 0.5 gms. for the sheep. Cattle have been reported to sicken on alfalfa containing 40 to 45 p.p.m. arsenic (Phillips, 1956). Arsenic is chemically rather indestructible. It can be detected in the liver and other tissue years after death and burial (Smith and Jones, 1957).

After lead, arsenic constitutes the most important toxicological hazard to farm animals. In the roasting of many metallic ores, arsenic may be carried as a dust with the smoke to contaminate neighboring livestock feed (Garner, 1957).

The relationship of the ingestion of arsenic in its various soluble, insoluble, organic, and inorganic forms by farm animals in various livestock feeds to the p.p.m. in the tissues and milk of dairy cows needs research and evaluation. Jones (1954) reported quantities of arsenic toxic to man in the milk of cows feeding on vegetation that had been sprayed with an arsenical weedkiller. Scheidy, et al., (1953) have shown that arsenic accumulates in the liver and is gradually distributed to other tissues. These workers found 2.1 p.p.m. in the liver of swine 7 days following the oral administration of 104 mg. of sodium arsanilate.

Fluorine is an important industrial contaminant of livestock feeds. Contamination is due to industrial air pollution settling on vegetables or to use of raw or nondefluorinated rock phosphate in mineral feeds.

During 1956, damage in court cases in the United States because of losses from fluorine amounted to over $50 million (Butler, 1958). It was a national problem involving many States of the Nation. In July 1959, the Utah State University sponsored a research seminar on the effects of fluorides on animals and man. In April 1960, the University of Wisconsin held a meeting on "The Review of the Research Work on Bovine Fluorosis."

The American College of Veterinary Toxicologists of the American Veterinary Medical Association is just finishing a color film strip with a documented descriptive narration on "A Study of Chronic Fluorosis in Livestock." This film was prepared with the assistance of many States, countries, and industrial companies that have conducted and financed research on the subject. Huffman (1952) and later Binns (1956) summarized the principal problems of industrial fluorine contaminations of livestock feeds.

Chronic fluorosis develops in farm animals receiving more than a minute amount of fluorine for a long time. The minimal amount of fluorine in the form of soluble fluorides required to produce injury in cattle and other farm animals lies between 1 and 2 mg./kg. body weight daily. The effect on a given animal depends on the species, the amount of fluorine ingested, the age during ingestion, the form of fluorine in the feed, and such stress factors as nutritional balance. All these factors must be considered in evaluating the effect of fluoride on an animal. Thus it is difficult to state the exact degree of herbage contamination that is significant. Contamination of pasture plants with industrial fumes and dusts to the extent of 25 to 50 p.p.m. on a dry-matter basis is generally considered to be potentially dangerous to grazing animals (Garner, 1957). However, in a 6-year study on a controlled experiment with healthy cattle on adequate calcium, phosphorus, protein, and vitamins, a total intake of 60 parts per million of sodium fluoride was required to produce definite chronic fluorosis (Newell and Schmidt, 1958). Other workers have classified the effects of ingestion of fluorine according to reduced feed consumption, failure to grow, loss in weight, lowered milk production, lowered reproduction, lameness, bone exostoses, fluorine content of bone, blood studies, urine fluorine, and teeth changes. The p.p.m. ingested have been correlated with these changes (Hobbs, et al, 1954). An increased intake of fluorine, environmental or experimental, results in a progression of dental effects. The changes from minor to severe effects in dental fluorosis are chalkiness of the enamel staining of the caries and erosion of the enamel, hypoplasia of the enamel, and hypoplasia of the teeth. These have all been excellently demonstrated in color plates by Hobbs, et al. (1954). In this work, it took about 2-1/2 years for cows on 50 p.p.m. fluorine to show reduced feed consumption and 18 months for cows on 70 p.p.m. This definite proof of the long time required to show illness and the detrimental effects of the prolonged feeding of small amounts of fluorides emphasize the need for much toxicological research on farm animals for effects of some chemicals in their feed.

Fluorides are emitted in various processes such as the manufacture of aluminum, steel, phosphate fertilizer, bricks, pottery, ferroenamel; also, in the calcining of iron ore where the source is mainly the fluorine-rich ore itself, from fused tricalcium phosphate furnaces, and from the burning of some coal. The troubles from fluorine effluents from steel plants and metal works usually have been when large amounts of the fluospar in the flux are used. The stack gases from phosphate fertilizer plants contain considerable quantities of fluorides. It is more difficult to control the emanations from phosphate fertilizer plants than from aluminum reduction plants.

Hay and forage crops may be contaminated from phosphatic dusts or from air-borne industrial effluents not properly controlled. Phosphatic soils may contain 50,000 p.p.m. and raw rock phosphate may be a constitutent of feed supplements. Hydrogen fluoride and fluorine are important air contaminants even in their extremely low concentrations of 0.001 to 0.10 p.p.m. by volume because they accumulate in the leaves of plants and can be absorbed in forage and hay to such an extent as to cause fluorosis in animals (Katz, 1956). Concentration values are lower than those of virtually all other pollutants, which may occur in the atmosphere from industrial activities; the various fluorides dispersed as aerosols have received considerable and particular attention because of the poisoning from such small amounts if consumed for a long time. Rupp (1956) reported the maximal permissible concentration of hydrogen fluoride for industrial operations is 3 p.p.m. Ferguson (1960) reports that industry as a whole is working very hard to clean up their atmospheric effluents. One aluminum reduction plant in particular has already spent in excess of $2-1/2 million in this particular field.

The fluorine content of any mineral or mineral mixture that is to be used directly for feeding of domestic animals shall not exceed 0.3 percent for cattle; 0.35 percent for sheep; 0.45 percent for swine and 0.6 percent for poultry. These limits have been set by the Association of American Feed Control Officials (Maynard and Loosli, 1956).

In normal bones, the fluorine content varies greatly and may reach a level of 1,200 p.p.m. or more on a fat-free dry basis. The bones of animals exhibiting a fluoride content of more than 4,500 p.p.m. may occur in bones that show lesions of fluorosis (Binns, 1956). The bones of animals showing clinical symptoms contain over 5,000 p.p.m. of fluorine and values as high as 20,000 p.p.m. are frequently found (Garner, 1957). Urine values are to be used in conjunction with other data and several samples are best for diagnostic significance (Hobbs, et al., 1954). Levels in the range of 10 to 20 p.p.m. indicate excessive fluorine ingestion and/or bone storage which are not sufficient to cause damage to the animal. The finding of urinary fluorine levels above 20 p.p.m. indicates excessive ingestion, which could cause interference with normal body function (A.C.V.T., 1960). During the long period of feedings when the animal shows no clinical symptoms the fluorine is mostly being deposited in the skeleton. When fluorine is no longer mobilized the animal adapts itself to more efficient excretion in the urine.

Nitrogen trichloride (Agene) has been used as a bleaching agent in the production of white flour from wheat. Because of its toxic properties in dogs, its use in the United States was made illegal about 1949 and in Great Britain in 1956.

Research showed that wheat flour "improved" with nitrogen trichloride produced the same symptoms in dogs (Mellanby, 1946). The toxic substances produced by nitrogen trichloride in flour was isolated from agenized zein (Bentley, et al., 1949), and independently from treated wheat flour (Campbell, et al., 1951). It is reported to be methionine sulphoximine, which interferes with glutamic acid metabolism in brain tissue (Pace and McDermott, 1952). Degenerative changes in the cerebral cortex and cerebellum with the nerve cells showing necrosis, shrinking, distortion, loss of substance, and eventual disappearance were described in dogs poisoned with agenized flour (Lewey, 1950). Nontoxic bleaching agents, particularly chlorine dioxide, have now largely replaced nitrogen trichloride.

One of the big problems with some livestock feeds has been the chronic toxicity of cottonseed cakes and meals from solvent extractions of some cottonseed. There is a specific toxic substance, gossypol, present in the pigment glands of cottonseed (Withers and Carruth, 1916). In methods of commercial extraction, most of the toxic gossypol is changed into bound gossypol which is much less poisonous. Cottonseed cakes and meals once varied considerably in their chronic toxicity, depending upon the method used for extracting the oil, the solvent used, and the variety of the seed. Symptoms may not be seen for some time after feeding. At low dietary levels, there was a loss of appetite and inhibition of growth. At higher levels, cattle, calves, and pigs manifest gastroenteritis, listlessness, and difficult breathing, followed later by convulsions and death (Garner, 1957).

Industry endeavors to keep the gossypol at low 0.02 to 0.04 percent. Because of variations in temperature with the hydraulic-press and of undesirable solvent in other processes, this amount may be greatly exceeded (Smith and Jones, 1957). Swine are more susceptible to poisoning from the toxic meal than other species. Symptoms commonly develop after pigs have been fed excessive amounts from 1 to 3 months. The lesions in swine are "white muscles," liver necrosis, edema, and cardiac changes (Smith, 1957). Chronic toxicity for gossypol exists even for adult cows. There are reports giving the usual expected variations in susceptibility of individuals, breeds, and species including adult cattle, calves, swine, sheep, dogs, and poultry (Leighton, et al., 1953). There are animals dying daily in the country that are being fed cottonseed meal, which show the same symptoms and lesions as have been proved as being produced by gossypol poisoning from these products. Laboratory findings are negative for those bacterial, viral, and chemical agents for which there are any diagnostic tests available.

The ARS well aware of the gossypol toxicity through their Southern Utilization Research and Development Division has conducted and contracted research on solving this problem. They developed a method by which nontoxic cottonseed meal can be commercially produced. The Animal Husbandry Division, ARS, fed growing pigs for 16 weeks on such feeds containing only 0.012, 0.018, and 0.024 percent of the free form of gossypol

supplied through cottonseed meals containing various amounts of this ingredient. No symptoms or lesions of gossypol poisoning were produced, and growth rate was not retarded (U.S.D.A., A.R.S., 1952). The method of producing low amounts of free gossypol made the feed satisfactory. Some cottonseed meals were found toxic. They produced death from free gossypol poisoning.

Nonruminants, young ruminants, and poultry are most susceptible to gossypol poisoning. The ARS Southern Utilization Research and Development and the Animal Husbandry Research Divisions and certain contracts for research did much on the problem. They developed analytical methods of determining the amounts of free or toxic gossypol in feeds; by use of dilutions of cottonseed meal containing 0.04 percent free gossypol and feeding tests on chickens. They determined that 0.016 percent free gossypol was the maximal safe level that could be included in the total diet for starting and growing poultry without harmful effects. Cottonseed meals of low free gossypol are now being made.

It was established in 1916 that trichloroethylene-extracted soybean oil meal was toxic to cattle, but that other meals produced by extraction with other chemical solvents were harmless (Stockman, 1916). A few years after it was first proved in Europe to be poisonous, extensive losses from 1923 to 1925 were experienced in Germany and Holland from this poisoning. Many reports showed that heavy losses from soybean meal extracted with trichloroethylene occurred in Iowa (1947-1952), Italy (1948), Colorado (1949), Kansas (1951), Minnesota (1951), Mississippi (1951), South Dakota (1951), Japan (1951), North Dakota (1952), and Hawaii (1952). A hemolytic toxic substance was demonstrated in the meal (Twiehaus and Leasure, 1951). In Minnesota, 38 of the herds studied were dairy herds and 6 herds were feeder steers (Pritchard, et al., 1952). The Agricultural Research Service in cooperation with the Iowa and Minnesota State Universities conducted studies of the chemistry and extraction processes and on the toxicity of various experimental trichloroethylene-extracted meal (TCESOM) products for farm and laboratory animals. The TCESOM meal was voluntarily withdrawn from the American market by the manufacturers concerned in 1952, because some of it produced a form of aplastic anemia in cattle after continued feeding and had toxicity for sheep and horses.

The solvent extraction processes leave only about 1 percent oil in the meal whereas the older hydraulic process and expeller process leave about 4 percent oil in the meal. The hydraulic process and the expeller process do not produce a toxic meal for livestock. The solvent extraction process does not produce a toxic product if hexane is used instead of trichloroethylene. However, the hexane process is more expensive, it has the fire hazard and requires more labor.

Cattle, horses, and sheep are susceptible to the toxic effects of this type of extracted meal and show acute and chronic forms of aplastic anemia. Symptoms in cattle in the acute form appear after 1 to 9 months of feeding and consist largely of high fever, hemorrhage, and death in 5 to 10 days. In the chronic form, the animals gradually lose condition, become anemic and weak, and eventually die after a course of illness involving several months in which little or no hemorrhage occurs. Cows that received 1 to 3 pounds of the toxic meal daily have died. Calves that received 1/4- to 1/2-pound daily have also died. In some instances, it took 1 pound of the toxic meal daily and 1 to 1-1/2 years to produce symptoms and death. The toxic substance appears to be mostly in the water-soluble fraction. Residual trichloroethylene in the extracted meal is not responsible for the effects noted (Pritchard, et al., 1956). Sheep losses from this meal have been associated with lambing, surgery, and a "trigger mechanism" such as pneumonia or mechanical injury (Holm, et al., 1953).

Swine feedings have not shown the swine to be markedly affected. No ill effects were shown from limited feedings of weaned gilts through their period of growth, reproduction, and lactation, and the feeding of their offspring to market weight. However, this does not give sufficient proof that it is nontoxic to swine (Pritchard, et al., 1956).

In a ration containing 22 percent toxic meal fed baby chicks for 50 days there was a 26 percent mortality against a 4.6 percent mortality in birds fed the same amount of a meal processed differently (Eveleth, et al., 1953). In other chick feeding a loss of 10

percent in weight gain without other toxic effects was reported. Turkeys have not been found to be markedly affected when fed a ration for 8 weeks which contained 45 percent of a toxic meal.

Feedings of goats, guinea pigs, hamsters, rats, and mice have not shown them to be markedly affected except that some showed some depression of growth rate. None of these animals, however, were satisfactory for use as test animals for the study and the identification of the toxic material (Pritchard, et al., 1956).

The industrial production of a feed, which is toxic for one species of livestock and not for another species, creates dangerous hazards. An important suggestion has been made that the mechanism that produces a toxic substance in this type of chemical extraction of soybean may produce the same or a similar toxicant in other plants. This suggestion was made following a feed toxicity in cattle. The toxicity was similar to that caused by trichloroethylene-extracted soybean oil meal but was in a herd that had ingested no soybean meal nor bracken fern (Eveleth and Holm, 1953). This plant produces very similar symptoms and lesions of poisoning.

Although certain chemicals have a favorable effect on the efficiency of lubricants in the mechanization of feed production for livestock, they are finding their way into these feeds. Their effect on the composition of milk, meat, and eggs needs to be understood. Because of the possible toxicological effects of certain chemicals used in lubricants, research on the effects of their use needs to be pursued vigorously to provide the public with the facts regarding their effect and the residues they may produce. Lubricants are petroleum products. The specific chemical composition of lubricants are trade secrets known only to a few of the industrial personnel in key positions of each company. There are numerous chemicals used in them, and changes are being made in these chemicals in lubricants, new ones are being made to replace others, and new patents are being obtained. It has been proved that lubricants contaminate some livestock feeds. So far as we know, these lubricants and air pollutants are not causing any livestock illness or death.

Cancer and other tumors are frequently found in farm animals. The Animal Disease and Parasite Research Division has had a small project on this subject for many years (Anderson, 1958; Davis, 1939, 1952; Monlux, Anderson, and Davis, 1956, 1957; Monlux and Davis, 1953; Sullivan and Anderson, 1958, 1959). In some of these publications malignant lymphomas of cattle were reported to occur frequently in this country and some recent reports from the Meat Inspection Division indicate they may be increasing. The cause is not known. Livestock feed must undoubtedly get contaminated with carcinogens from air pollution because it is impossible to keep livestock hay, grass, and other forage from exposure to the air. Some coal tar and petroleum compounds have been found as air pollutants which have also been used in experimental production of carcinogenic effects in animals (Kotin, 1958). A recent publication in Cancer Research 1959, 19:321-325 (1959) reports on the experimental finding of a carcinogenic agent in the urine of cattle which is a metabolite of the essential amino acid tryptophan. The British Journal of Cancer 11:212-228 (1957) gives a report on cancer of the urinary bladder induced in mice with metabolites of aromatic amines and tryptophan.

Highly chlorinated naphthalene poisoning of livestock feeds was another industrial contamination in the United States from 1941 to 1953. During this period, there was an enormous loss of cattle by sickness and death from a disease of unknown cause, called X-disease, or bovine hyperkeratosis. Losses became particularly large in some States such as Illinois, Oklahoma, Tennessee, Texas, and Wisconsin (USDA, 1953). The Texas Agricultural Experiment Station reported that they estimated 100,000 head of cattle were affected in Texas and Oklahoma during the spring of 1953 (Schmidt, et al., 1958). Research investigations by the Agricultural Research Service was instigated in 1947 because of the rapidly increasing importance of X-disease, or hyperkeratosis of cattle, which existed in 27 States. Scientists in 18 States in 1949-1953 conducted cooperative research with the Agricultural Research Service to find the cause. They had to study the whole environment of the affected cattle--soils, plant life, fertilizers, bacterial flora of the rumen, viruses, and livestock feeds. The cooperation and contributions made by many research workers

were outstanding and their contributions are too numerous to name and to cite in a paper of this length and nature. The results of research have been covered briefly but completely in chronological order (Gibbons, Lee, Johnson, and Robinson, 1949; Lee, 1952; Udall, 1954; Lee, 1956). The symptoms and lesions also cannot be given for lack of time, but they are described and illustrated in publications (USDA, 1954; USDA, 1959; Udall, 1954).

When it was proved that some bread crumbs being fed to cattle were toxic A.R.S. reported it to the Food and Drug Administration on July 12, 1951. When it was also later proved that the milk of cows fed these bread crumbs would kill the calf this was also reported to the Food and Drug Administration. They made extensive investigations and did everything possible, but the cause of the toxicity, or the chemical or the important commodity concerned was not known. The only tests to show toxicity was the bovine, through its peculiar symptoms. The bakeries concerned sold no more crumbs and changed about everything they were using. All parties concerned agreed it would be best not to inform the public about the bread and milk being poisonous as it would not help and nothing could be done about it that was not already being done.

By February 1952 it had been proved in this research that a certain pelleted alfalfa feed, a particular batch of bread crumbs from the slicer, a certain timothy hay, a German wood preservative, and a lubricant would produce the disease. In February 1952, Agricultural Research Service set up an experiment at Beltsville, Md., in its Animal Husbandry Research Division, which proved that lubricants for greasing pellet machines went into the pellets.

It has been proved that highly chlorinated naphthalene is the only cause of the specific cattle entity with which the research was concerned and that the greatest amount of it was being produced by the feeding of pellets contaminated with lubricants containing highly chlorinated naphthalene to lubricate the pellet machines. The Department issued a warning in March 1953 and judging from results obtained this chemical has not been in the lubricants used on pellet machines since. If so, it has been a lower percentage of the chemical than in the 3 percent and the 10 percent formerly used, or it has been of lower chlorination than tetrachloronaphthalene.

Only by research can better ways be found than the trial and error methods of the past in learning about industrial chemical contamination of livestock feed. None of these severe livestock losses have been the fault of livestock feed companies, or intentional on the part of industry. They have been due to a lack of research knowledge on the part of all concerned.

REFERENCES

1. Anonymous 1951. Nutrition Review, 9-284.

2. Allcroft, R. 1951. Lead poisoning in cattle and sheep. Am. J. Vet. Res. 89.589-590.

3. Allen, M. J., and Boyland, E. 1957. Cancer of the urinary bladder nduces in mice with metabolites of aromatic amines and tryptophane. British Jour. Cancer. 11:212-228.

4. American College of Veterinary Toxicologists. 1960. A study of chronic fluorosis in livestock. A documented film strip. Am. Vet. Med. Assoc.

5. Anderson, W. A., and Davis, C. L. 1957. Neoplasms of the genitalia of the bovine. Proceedings - Symposium on Fertility and Reproduction. Colorado State University. pp. 41-49.

6. Anderson, Wayne A., Monlux, A. W., and Davis, C. L. 1958. Epithelial tumors of the bovine gallbladder--A report of eighteen cases. Am. J. Vet. Res. 19:58-65.

7. Bentley, H. R., McDermott, E. E., Pace, J., Whitehead, J. K., and Moran, T. 1949. Nature (London). 164:438.

8. Binns, W., 1956. Chemical poisoning. Yearbook of Agric. Separate No. 2671.

9. Binns, W., and Beeson, K. C. 1959. Unpublished data.

10. Blaxter, K. L. 1950. Lead as a nutritional hazard to farm livestock. II. The absorption and excretion of lead by sheep and rabbits. Jour. Comp. Path. 60:140-159.

11. Butler, Charles. 1958. Impact of air pollution on our agricultural considerations. Proceedings--National Conference on Air Pollution. Nov. 18-20, USPHS, 251-255.

12. Campbell, P. N., Work, T. S., and Mellanby, E. 1951. The isolation of a toxic substance from agenized wheat flour. The Biochemical Jour. 48:106-113.

13. Davis, C. L. 1939. Primary carcinoma of the lung of a sheep. Am. J. Vet. Res. 94:237-240.

14. Davis, C. L., Phillips, L. R., and Neubuerger, K. T. 1948. Malignant meningioma in a dog. Am. J. Vet. Res. 112:367-370.

15. Davis, C. L., and Shorten, H. L. 1952. Carcinoma of the eye of sheep. Am. J. Vet. Res. 121:20-24.

16. Eveleth, D. F., and Goldsby, A. I. 1953. Toxicosis of chickens caused by trichloroethylene-extracted soybean meal. Am. Vet. Med. Assoc. 123:38.

17. Eveleth, D. F., and Holm, G. C. 1953. Feed toxicity similar to that caused by trichloroethylene-extracted soybean oil meal. Am. Vet. Med. Assoc. 122:377.

18. Furgeson, H. E. 1960. Personal communication, March 11, 1960. Anaconda, Montana.

19. Garner, R. J. 1957. Veterinary Toxicology. Williams and Wilkins Co., Baltimore, Md.

20. Gibbons, W. J., Lee, A. M., Johnson, A. G., and Robinson, W. O. 1949. Report of the Preliminary Survey on X-Disease (Hyperkeratosis) of Cattle. U. S. Dept. Agr. Mimeographed Report, Part I and Part II. February 1949.

21. Gobble, J. L. 1958. Feeds and feeding. Chapt. 51, Section VIII. Nutrition feeds and management. Diseases of swine. Edited by Howard W. Dunne. Iowa State College Press.

22. Hobbs, C. S., Moorman, R. P. Jr., Griffith, J. M., West, J. L., Merriman, G. M., Hansard, S. L., and Chamberlain, C. C. 1954. Fluorosis in cattle and sheep. Agri. Expt. Sta. Bull. 235. University of Tennessee.

23. Holm, G. C., Eveleth, D. F., and Dinusson, W. E. 1953. Trichloroethylene soybean meal poisoning in sheep. Am. Vet. Med. Assoc. 122:380.

24. Huffman, W. T. 1952. Effects on livestock of air contamination caused by fluoride fumes. U. S. Conference on Air Pollution, Louis C. McCabe, Chairman. McGraw-Hill Book Co. pp. 59-63.

25. Jones, L. M. 1954. Veterinary Pharmacology and Therapeutics. Ames, Iowa State College Press.

26. Kates, Morris. 1956. City Planning Industrial-Plant Location. Hydrogen ir Fluoride. Air Pollution Handbook by Magill, Holden, and Ackley. McGraw-Hill Book Co., New York.

27. Kotin, Paul. 1958. Air Pollution and Cancer. Experimental Tumor Production with Air Pollutants. Proceedings--National Conference on Air Pollution. Wash. D. C., Nov. 18-20, 1958. pp. 228-231. U.S.D.H.E.W.--Public Health Service.

28. Lee, Aubrey M. 1952. Our newer knowledge of bovine hyperkeratosis (X-disease). Proceedings--U. S. Livestock Sanitary Assoc. pp. 175-194.

29. Lee, Aubrey M. 1956. Hyperkeratosis. USDA Yearbook of Agriculture Animal Diseases. pp. 273-276.

30. Leighton, R. E., Anthony W. B., Huff, J. S., and Rupel, I. W. Jour. Dairy Sci. 3(:601.

31. Lewey, F. H. 1950. Neuropathological changes in nitrogen trichloride intoxication of dogs. J. Neuropath. and Expt. Neurol. 9:396-405.

32. Magill, Holden, and Ackley. 1956. Air Pollution Handbook. McGraw-Hill Book Co., New York.

33. Maynard, L. A., and Loosi, J. K. 1956. Animal Nutrition. pp. 160, 4th Edit. McGraw-Hill Book Co., Inc., New York.

34. Mellanby, E. 1946. British Medical Jour. 2:885.

35. Monlux A. W., Anderson, W. A., and Davis, C. L. 1956. A survey of tumors occurring in cattle, sheep, and swine. Am. J. Vet. Res. 17:646-677.

36. Monlux, A. W., Anderson, W. A., and Davis, C. L. 1957. The diagnosis of squamous cell carcinoma of the eye (cancer eye) in cattle. Am. J. Vet. Res. 18:5-34.

37. Monlux, A. W., and Davis, C. L. 1953. Multiple schwannomas of cattle (nerve sheath tumors; multiple neurilemmomas; neurofibromatosis). Am. J. Vet. Res. 14:499-509.

38. Newell, G. W., and Schmidt, H. J. 1958. Effects of feeding Fluorine to dairy cattle--A six year study, Am. J. Vet. Res. 19:363-375.

39. Pace, J., and McDermott, E. E. 1952. Nature (London) 169:415.

40. Panukcu, A. M., Brown, R. R., and Price, J. M. 1959. Tryptophane metabolites in the urine of Turkish cows with urinary bladder cancer. Cancer Res. 19:321-329.

41. Phillips, P. H. 1956. Effects of air pollutants on farm animals. Air Pollution Handbook. Magill, Holden, and Ackley. McGraw-Hill Book Co., New York.

42. Pritchard, W. R., Hammer, R., Sautter, J. H., and Schultze, M. O. 1956. Susceptibility of the horse to the toxic factor in trichloroethylene-extracted soybean oil meal. Am. J. Vet. Res. 17:441-443.

43. Pritchard, W. R., Mattson, W. E., Sautter, J. H., and Schultze, M. O. 1956. The use of young calves for study of various aspects of toxicity of trichloroethylene-extracted soybean oil meal. Am. J. Vet. Res. 17:437-441.

44. Pritchard, W. R., Mattson, W. E., Sautter, J. H., and Schultze, M. O. 1956. Failure to demonstrate the presence of a toxic factor in the milk of cows fed toxic specimens of trichloroethylene-extracted soybean oil meal. Am. J. Vet. Res. 17:444-445.

45. Pritchard, W. R., Perman, V., Mattson, W. E., Sautter, J. H., and Schultze, M. O. 1956. The effects of feeding trichloroethylene-extracted soybean oil meal to sheep. Am. J. Vet. Res. 17:446-448.

46. Pritchard, W. R., Sauer, F., Rehfeld, C. E., Perman, V., Sautter, J. H., Wada, S., and Schultze, M. O. 1956. Observations with laboratory animals fed trichloroethylene-extracted soybean oil meal. 17:448-453.

47. Pritchard, W. R., Rehfeld, C. E., Mattson, W. E., Sautter, J. H., and Schultze, M. O. 1956. The effect of feeding different levels of trichloroethylene-extracted soybean oil meal to young heifers--Experimental production of chronic aplastic anemia. Am. J. Vet. Res. 17:430-437.

48. Pritchard, W. R., Rehfeld, C. E., Mizuno, N. S., Sautter, J. H., and Schultze, M. O. 1956. Studies on trichloroethylene-extracted feeds. I. Experimental production of acute aplastic anemia in young heifers. Am. J. Vet. Res. 17:425-429.

49. Pritchard, W. R., Rehfeld, C. E., and Sautter, J. H. 1952. Aplastic anemia in cattle associated with ingestion of trichloroethylene-extracted soybean oil meal. Am. Vet. Med. Assoc. 121:1-9.

50. Rupp, Walter H. 1956. Major air contaminants. Air Pollution Handbook by Magill, Holden, and Ackley. McGraw-Hill Book Co., New York.

51. Scheidy, S. F., Wilcox, P. W., and Creamer, A. A. 1953. Am. J. Vet. Med. Assoc. 123:341.

52. Schmidt, Hubert, and Franklin, T. E., 1958. Hyperkeratosis investigations in Texas, 1946-56. Texas Agr. Expt. Sta. M. P. 316., Nov. 1958.

53. Smith, H. A. 1957. Pathology of gossypol poisoning. Am. J. Path. 33:353-365.

54. Smith and Jones. 1957. Veterinary Pathology. Lea and Febiger, Phil.

55. Steimer, P. E., and Bengston, J. S. 1951. Research and economic aspects of tumors in food-producing animals. Cancer--Med. Book Dept., Harper and Bros.

56. Stockman, S. 1916. Poisoning in cattle on meal from soybean after extraction of the oil. Jour. Comp. Path. and Ther. 29:95-107.

57. Sullivan, D. J., and Anderson, W. A. 1958. Tumors of the bovine acoustic nerve. Am. J. Vet. Res. 19:848-852.

58. Sullivan, D. J., and Anderson, W. A. 1959. Embryonal nephroma in swine. Am. J. Vet. Res. 20:324-332.

59. Twiehans, M. J., and Leasure, E. E. 1951. The presence of a hemorrhagic factor in soybean pellets extracted with trichloroethylene as a solvent when fed to cattle. Am. J. Vet. Med. Assoc. 46:428-431.

60. Udall, D. H. 1954. The practice of veterinary medicine. 6th Rev. Edit. pp. 769-773. Published by the Author. Ithaca, New York.

61. U.S.D.A.-A.R.S. Report of the Chief, Bureau of Animal Industry. 1952, pp. 33; 1953. pp. 52-53.

62. U.S.D.A.-A.R.S. 1954. Hyperkeratosis (X-disease) of cattle. U. S. Dept. Agr. Leaflet 355 March 1954.

63. U.S.D.A.-A.R.S. 1957. Improved cottonseed meal for broiler and chick rations. Southern Utilization and Animal Husbandry Research Division, ARS. Special Report 22-40.

64. U.S.D.A.-A.R.S. 1959. Hyperkeratosis of cattle. U. S. Dept. Agr. Leaflet 447. June 1959.

65. White, E. G., and Cotchin, E. 1948. Natural and Experimental cases of poisoning of calves by flaking lead paint. Vet. J. 104:75-91.

66. Withers, W. A., and Carruth, F. E. 1916. Gossypol, the toxic substance in cottonseed meal. J. Agr. Res. 5:261.

INSECTICIDE RESIDUES IN PLANTS, ANIMALS, AND SOILS

W. E. Westlake and J. P. San Antonio[1]

It is impossible to give anything approaching a comprehensive discussion of our knowledge of insecticide residues in a paper as brief as this one must be. It has been necessary, therefore, to select a few examples illustrating the behavior of certain representative types of insecticides. The discussion will be limited to insecticides, although much of the chemistry involved in determining the amounts present and the fate of these compounds applies equally to other types of pesticides.

Various chemical and physical factors influence the rate of degradation and the amounts of a parent compound and related products that may be present at any given time. On plant surfaces and in soils, temperature, light, humidity, air movement, volatility of the compounds, and microorganism activity are important factors influencing the breakdown and mechanical dispersion of residues. The action of enzymes is probably the most important single factor within plants and animals.

Since the disappearance of insecticide residues is of the utmost importance, particularly on plants, the present discussion will deal principally with some of the chemical changes that have been observed or proposed. Progress in research on metabolites of non-nutritive substances in general is graphically illustrated by a comparison of the first and second editions of R. T. Williams' "Detoxication Mechanisms" (19). The first edition contained 288 pages and the second, published 12 years later, in 1959, contains 796 pages. During this same period significant advances have similarly been made in our knowledge of the occurrence and fate of residues following the application of insecticides.

When an insecticide is applied to plants, animals, or soils, there are many factors that may influence chemical changes and the rates of such changes depend upon the nature of the compound and the particular conditions to which it is subjected. The chemical reactions that occur may produce compounds that are either less or more toxic than the one applied.

CHLORINATED HYDROCARBON INSECTICIDES

DDT has been the subject of comprehensive studies to determine its rate of degradation and the products formed. Fleck and Haller (8) studied the dehydrochlorination of this insecticide and found that iron and aluminum salts catalyzed dehydrochlorination under

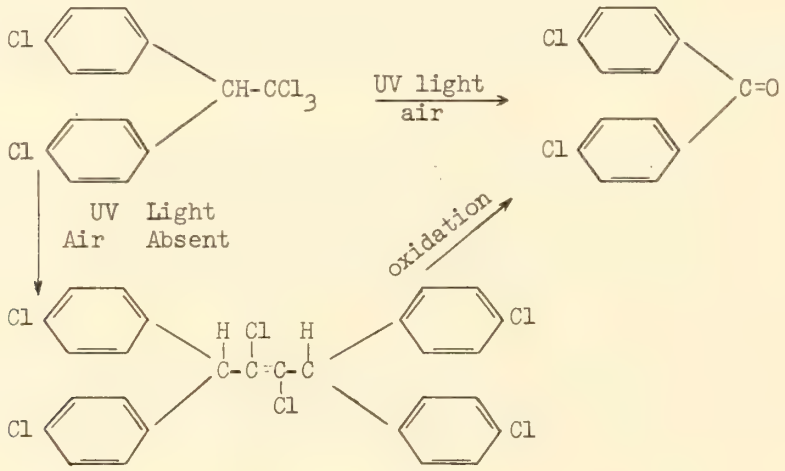
---

[1] Chemist, Entomology Research Division, and Plant Physiologist, Crops Research Division, respectively, Agricultural Research Service, U. S. Department of Agriculture.

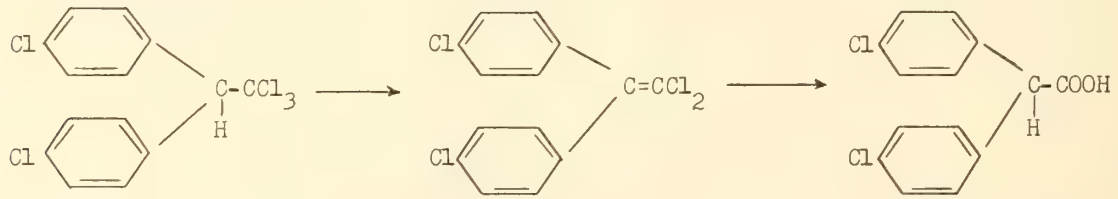
certain conditions. The reaction produces 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE) as follows:

DDT DDE

Fleck (7) found ultraviolet light to catalyze the decomposition of DDT. In the presence of air, one of the decomposition products is 4,4'-dichlorobenzophenone. When air is absent, 2,3-dichloro-1,1,4,4-tetrakis-(p-chlorophenyl)-2-butene is formed. This compound, through subsequent oxidation, may be converted into 4,4'dichlorobenzophenone. Thus:



In mammals 2,2-bis(p-chlorophenyl) acetic acid (DDA) has been identified and shown to be excreted in the feces and urine. The mechanism of formation of DDA is believed to be an initial dehydrochlorination to DDE, which is then hydrolyzed to DDA, as follows:



DDT DDE DDA

Mattson, et al. (14) found both DDT and DDE in all but 2 of about 50 samples of human fat. DDE represented 39-86 percent of the total. Walker, et al. (18), in a study of meals purchased in restaurants, found DDT and DDE to be present, at low levels, in every sample taken.

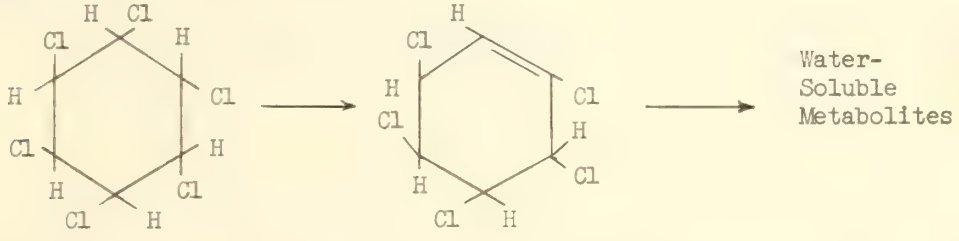
The only metabolite present in insects in any significant quantity is DDE, and no evidence of conversion to DDA has been found. Little is known about the fate of DDT in soil.

The closely related compound, TDE, is converted to its ethylene derivative in mammals. In some insects, however, this conversion does not take place and this lack of ability to metabolize TDE may be related to an increased toxicity to such insects.

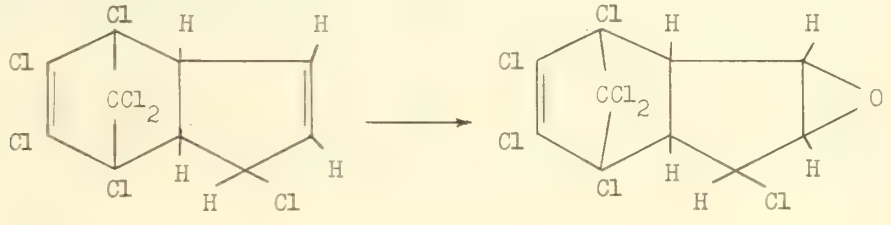
Benzene hexachloride has been found, by Sternberg and Kearns (17), to be converted to pentachlorocyclohexene by dehydrochlorination. San Antonio (16) isolated a metabolite from carrot plants which in some respects appeared similar to this compound.

The occurrence of metabolites of BHC and DDT in insects has recently been reviewed (3). In soils a fairly rapid loss of BHC takes place, but metabolites and degradation products have not been identified. The persistence and breakdown of chlorinated hydrocarbon insecticides in different soils has been recently reported (12, 13).

Steps in the degradation of BHC may be as follows:

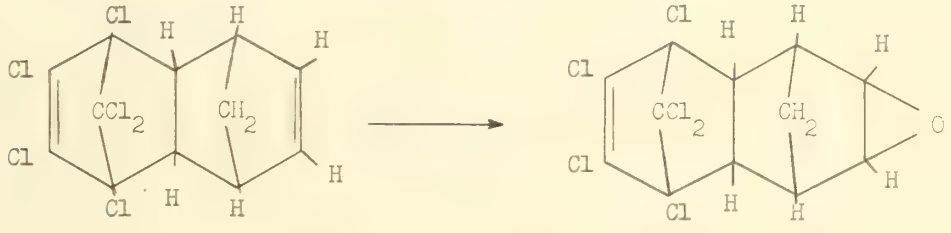


Davidow and Radomski (4, 5) found heptachlor epoxide as a metabolite of heptachlor in animals; this appears to be the first record of epoxidation of a foreign organic compound in an animal body. Ely, et al. (6) detected heptachlor epoxide in the milk of cows fed technical heptachlor, and Gannon and Bigger (9) found it in soil treated with this insecticide.



Heptachlor Heptachlor Epoxide

Bann, et al. (1) showed the conversion of aldrin to dieldrin, a second example of epoxidation, in animals, and showed storage of dieldrin in the fat. This conversion, too, has since been found to occur in plants and in soils (9, 10).



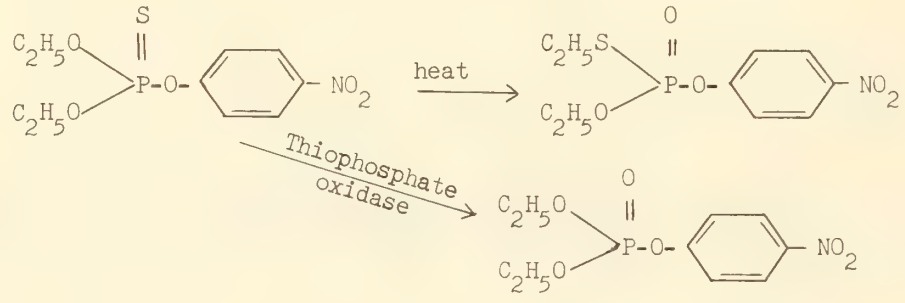
Aldrin Dieldrin

These two cases illustrate the conversion of insecticides to products that are more toxic to insects and are considered, by some, to be more toxic to man. The fate of the epoxides is not known, but it has been shown that they are more stable than the original materials. Recent studies also have indicated the presence of compounds other than heptachlor and heptachlor epoxide in plants after technical heptachlor has been applied. Investigations are now being made to identify these compounds.

The mechanism of the conversion to epoxides is not known, but it is thought that in soils microorganisms may be at least partly responsible. In animals the conversion appears to be practically complete, and takes place rapidly. Only traces of aldrin and heptachlor have been found in animal tissues following feeding of these compounds whereas relatively large amounts of their epoxides are formed and stored.

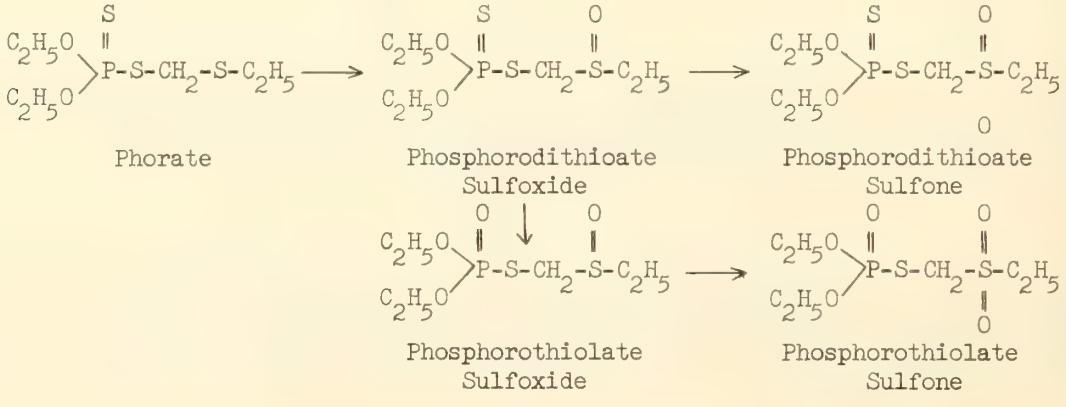
ORGANIC-PHOSPHORUS COMPOUNDS

The first of the organic-phosphorus insecticides to gain widespread use was parathion. This compound is converted to the S-ethyl isomer by heating. A more toxic compound, para-oxon, is formed by enzymatic action in plants, as follows:



In animals the additional products p-nitrophenol and p-aminophenol are also formed (11). Not much seems to be known regarding the decomposition products of parathion in soils.

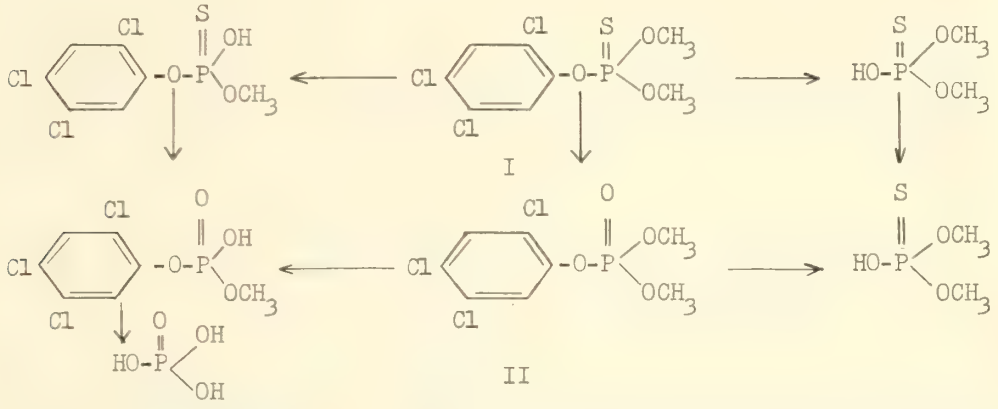
Phorate, another organic-phosphorus insecticide coming into widespread use, is rapidly metabolized in plants to sulfoxide and sulfone forms. Bowman and Casida (2) have found the metabolic pathway, in cotton, to be as follows:



In this instance phorate, a weak cholinesterase inhibitor, is converted into a very potent inhibitor, the phosphorothiolate sulfone. Phorate is very effective in plants grown

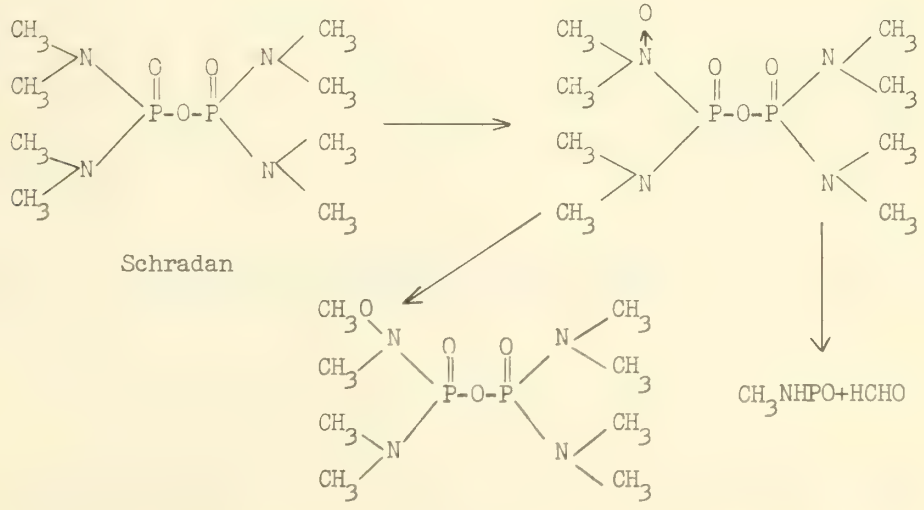
in soil treated with it, or from treated seed. Conversion to the sulfone has not been shown in the soil and may take place only after entry into the plant tissue.

The metabolism of ronnel (I) in cattle has been studied by Plapp and Casida (15), using P^{32} tagged material. Based on these studies, the following metabolic pathway is proposed:

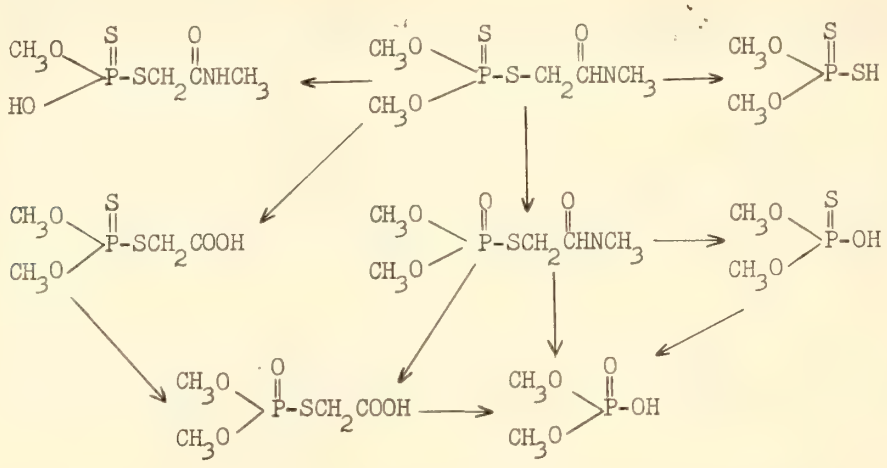


The oxygen analog (II) was not isolated as an in vivo metabolite, but the inhibition of blood cholinesterase in the cows indicated its presence. Ronnel is a very weak cholinesterase inhibitor compared with its oxygen analog. This reaction and hydrolysis at the phosphorus-oxygen bond has been shown for other dialkyl aryl phosphorothioates. Hydrolytic attack at the alkoxy group to yield a stable monoalkyl phosphorodiester had not been previously reported. The ultimate fate is probably degradation to phosphoric acid, which may be incorporated in phospholipids and phosphoproteins.

Schradan is another compound that is nontoxic until it has undergone oxidation in the plant tissues, to convert it into a very potent insecticide. This compound illustrates the oxidative demethylation of an N-methyl phosphoramide.



One of the new organic-phosphorus insecticides now being investigated is dimethoate. Studies of the metabolism of this compound, using P^{32} tagged material, have suggested the metabolic pathway shown:

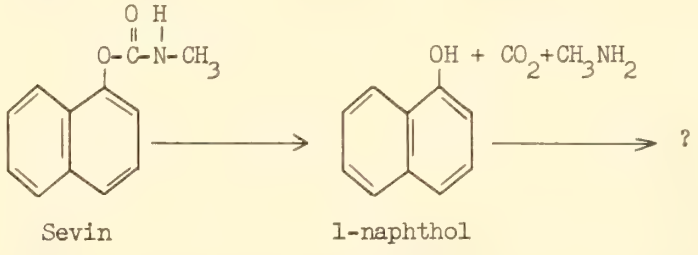


As observed in other compounds of this type, the oxygen analog is a more potent esterase inhibitor than the parent compound. However, the difference is not great, and none of the other metabolites inhibit cholinesterase.

The breakdown products appear to be the same in plants and animals. No information has been published on the fate of this compound in soils.

CARBAMATES

A recently developed carbamate, 1-naphthyl methylcarbamate (Sevin), is showing great promise for the control of many insects. It is interesting to note that several carbamates are used as plant-growth regulators. Sevin is hydrolyzed to 1-naphthol in plants and animals, but the 1-naphthol is not sufficiently stable to permit the detection of more than trace amounts. The metabolic process apparently starts with hydrolysis, thus:



The extent and rate of hydrolysis have not been determined, but the rate is apparently slow under most conditions. Under alkaline conditions, however, hydrolysis is rapid and complete.

PERSISTENCE OF INSECTICIDES

One of the chief items of interest in the study of insecticide residues is a study of their persistence under the conditions of use. To determine this, at appropriate intervals

the chemist analyzes samples of the product to which application has been made. He then plots the results to show the rate of loss. Such curves show the resultant action of the many factors influencing loss of the insecticide. Figures 1 and 2 show the curves for several chlorinated hydrocarbon insecticides in soil. Figure 3 shows the rate of loss of DDT from apples. These curves are typical for the more persistent insecticides. Figure 4, for Diazinon on grapes, illustrates the loss of a less persistent insecticide.

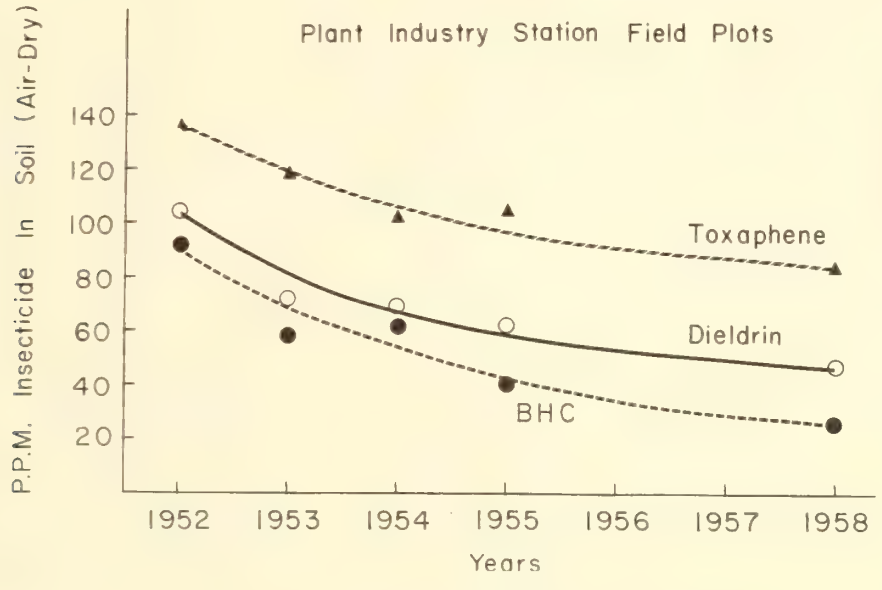


Figure 1.--Persistence of toxaphene, dieldrin, and BHC in soil.

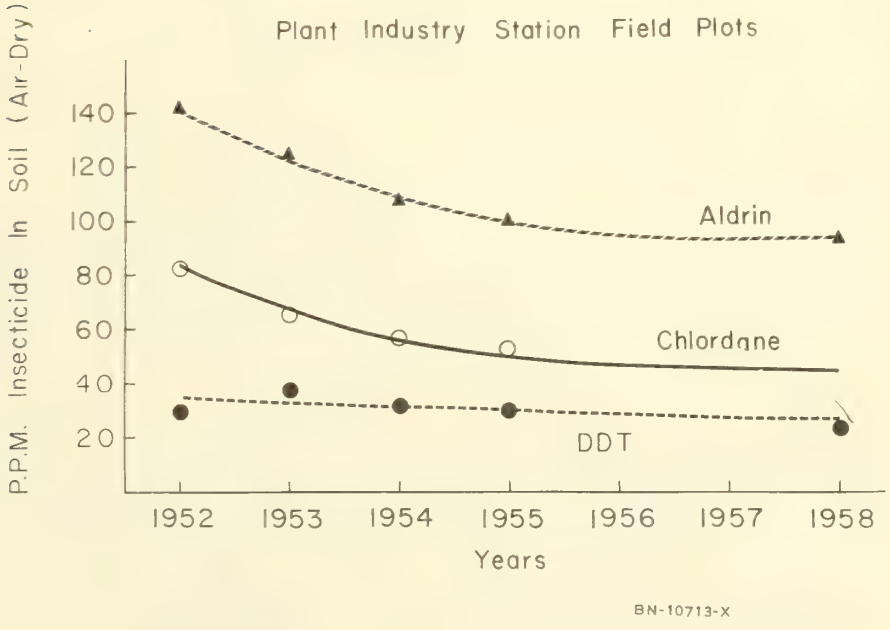


Figure 2.--Persistence of aldrin, chlordane, and DDT in soil.

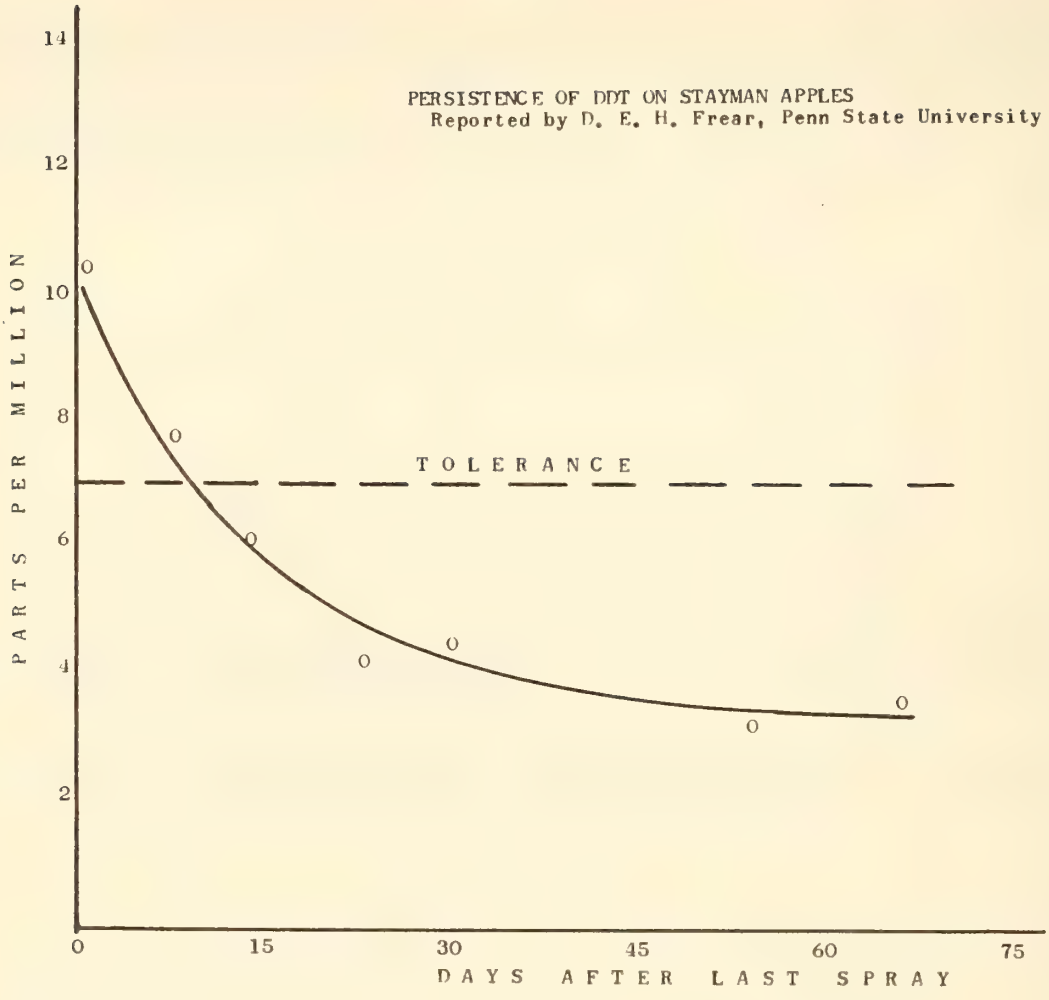


Figure 3.--Persistence of DDT on apples.

TRANSLOCATION OF INSECTICIDES

Some insecticides are known to enter the sap stream of plants and be translocated to parts remote from the point of application. One outstanding example is phorate, which may be taken up by the roots and translocated to the aerial parts of plants, where it kills insects feeding on them. All degrees of movement in the plant have been observed, ranging from the above example, to a very slight movement. The actual extent and mechanism of movement of the chlorinated hydrocarbon insecticides into the roots, tubers, and above-ground parts of plants is actively being studied at present.

It has been generally assumed that compounds having very low solubility in water would not be translocated in plants to any appreciable degree. Some data, many of them as yet unpublished, indicate that this may not always be true. San Antonio (16) has analyzed carrots grown in soil treated with BHC, using a paper chromatographic procedure, and reports the greatest accumulation of residues in the fibrous roots with smaller amounts in the edible part, stems, and leaves. Representative data are shown in Table 1. While the amount found in stems and leaves was small (0.25 p.p.m.), it does indicate possible translocation and the need for further study. Recent biological observations by entomologists have also shown the presence of insecticidally effective compounds in the aerial

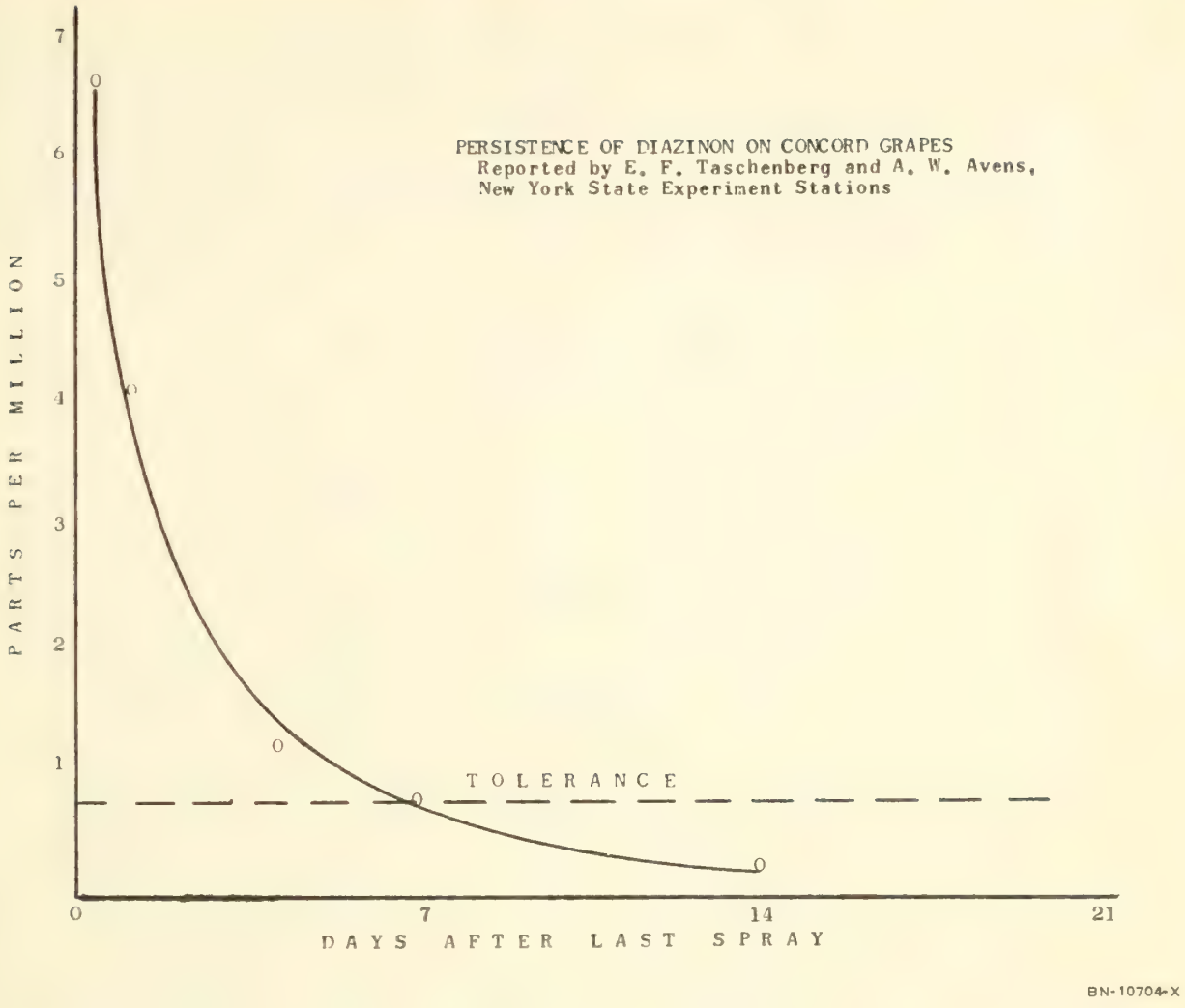


Figure 4.--Persistence of Diazinon on grapes.

parts of plants grown in soil treated with heptachlor. Studies are underway to obtain more data concerning this very important problem.

The foregoing examples point out the necessity for developing analytical methods that are capable of detecting minute quantities of insecticides and the various metabolites or degradation compounds that may occur. Colorimetric procedures, radioactive tracers, paper chromatography, infrared spectroscopy, and gas chromatography are some of the approaches used by chemists. There is probably no field of insecticide research that offers a greater opportunity for valuable contributions.

Our situation regarding knowledge of the occurrence and the fate of insecticide residues, then, is briefly this. We have accumulated a wealth of new data during the past decade, and have increased our understanding of the degradation of residues manyfold. This is particularly noteworthy in view of the limited support that has been given such research. Despite this progress, we are still much too weak in our understanding of the metabolism and degradation of these residues. We are little more than holding our own as more compounds come into use. Intensified programs of study to find the route of degradation or metabolism of insecticides, including many of those with long-established uses, is essential if we wish to keep our research on a scientifically sound basis.

TABLE 1.--Lindane in Treated-Plant[a] Tissues

Age of plants at harvest (days)	Plant tissue, analyzed	No. of different tissue extracts, analyzed[b]	Fresh weight of tissue, grams	Treatment of extract[c]	Av. of lindane estimations (p.p.m.)
	Carrot, *Daucus carota*, var. Imperator (10 p.p.m.)				
100-120	Fibrous roots	4	1	C and D	25
	Edible portion	4	40	C	3
	Stem and leaf	1	57	C	0.25
	Carrot, *Daucus carota*, var. Imperator (5 p.p.m.)				
53	Fibrous roots	2	7	D	25
120	Do	2	2	D	17
	Edible portion	2	40	C	0.25
	Snap bean, *Phaseolus vulgaris*, var. Topcrop (5 p.p.m.)				
50	Edible beans	1	50	C	0
60	Fibrous roots	1	3	D	3
	Tomato, *Lycopersicon esculentum*, var. Rutgers (5 p.p.m.)				
90	Fibrous roots	1	16	C	1.5
	Sweet potato, *Ipomoea batatas*, var. Porto Rico (5 p.p.m.)				
120	Fibrous and edible roots	2	40	C	-0.5
	Wheat, *Triticum aestivum*, var. Atlas 66 (5 p.p.m.)				
70	Fibrous roots	1	24	C	1
	Potato, *Solanum tuberosum*, var. Katahdin (5 p.p.m.)				
30	Fibrous roots	1	10	D	5
120	Do	2	2	D	2.5
	Potato	1	40	C	0.5
	Potato peel	1	0.7	D	0.0
	Corn, *Zea mays*, var. Golden Bantam (1 p.p.m.)				
70	Roots	1	20	C	0

[a] Plants grown in soil containing lindane. Soil concentration of lindane given in parentheses.

[b] At least two aliquots of each tissue extract chromatographed.

[c] D. Initial extract of tissue applied directly to spot line of chromatographic paper.
C. Extract cleaned up before chromatography.

ACKNOWLEDGMENTS

Figures 1 and 2 were taken from unpublished data from cooperative studies by Crops Research Division and Entomology Research Division, Beltsville, Md.

Figures 3 and 4 were taken from an unpublished report of Cooperative Regional Project NE-36; the investigators were D. E. H. Frear, Pennsylvania State University and E. F. Taschenberg and A. W. Avens, New York Experiment Station, Geneva, New York, respectively.

LITERATURE CITED

(1) Bann, J. M., DoCino, T. A., Earle, N. W., and Sun, Yun-Pei. Jour. Agr. Food Chem. 4, 937 (1956).

(2) Bowman, J. S., and Casida, J. E. Jour. Agr. Food Chem. 5, 192 (1957).

(3) Bradbury, F. R., and Standen, H. Jour. Sci. Food and Agr. 11, 92 (1960).

(4) Davidow, B., and Radomski, J. L. Jour. Pharmacol. 107, 259 (1953).

(5) Davidow, B., and Radomski, J. L. Ibid. 266.

(6) Ely, R. E., Moore, L. A., Hubanks, P. E., Carter, R. H., and Poos, F. W. Jour. Dairy Sci. 38, 669 (1955).

(7) Fleck, E. E. Jour. Am. Chem. Soc. 71, 1034 (1949).

(8) Fleck, E. E., and Haller, H. L. Jour. Am. Chem. Soc. 66, 2095 (1944).

(9) Gannon, N., and Bigger, J. H. Jour. Econ. Ent. 51, 1 (1958).

(10) Gannon, N., and Decker, G. C. Jour. Econ. Ent. 51, 3 (1958).

(11) Gardocki, J. F., and Hazleton, L. W. Jour. Amer. Pharm. Assn. 40, 491 (1951).

(12) Lichtenstein, E. P., and Schulz, K. R. Jour. Econ. Ent. 52: 118 (1959).

(13) Lichtenstein, E. P., DePew, L. J., Eshbaugh, E. L., and Sleesman, J. P. Ibid 53: 136 (1960).

(14) Mattson, A. M., Spillane, J. T., Baker, C., and Pearce, G. W. Anal. Chem. 25, 1065 (1953).

(15) Plapp, F. W., and Casida, J. E. Jour. Agr. Food Chem. 6, 662 (1958).

(16) San Antonio, J. P. Jour. Agr. Food Chem. 7, 322 (1959).

(17) Sternberg, J., and Kearns, C. W. Jour. Econ. Ent. 49, 548 (1956).

(18) Walker, K. C., Goette, Mary B., and Batchelor, G. S. Jour. Agr. Food Chem. 2, 1034 (1954).

(19) Williams, R. T. Detoxication Mechanisms. 2nd ed., 796 pp. John Wiley & Sons, N. Y. (1959).

NEMATOCIDES IN PLANTS AND SOILS

A. L. Taylor[1]

The search for nematocides started more than 80 years ago, and one nematocide was in limited use before 1900. The first nematocide was placed on the market in the United States in 1937. This was followed by another about 1940, both suitable for only limited use in greenhouses and vegetable seedbeds. The rapid advance in the use of nematocides started about 1946 when materials suitable for field use became generally available. Use has grown to over a half million acres in 1959. Most of the treated land has been planted to tobacco, vegetable, or pineapples, though nematocides are used for a variety of other crops.

The chemicals most widely used as nematocides are ethylene dibromide (EDB), 1,2-dibromo-3-chloropropane (DBCP), and 1,3-dichloropropene, obtainable either as technical dichloropropenes, or mixed with 1,2-dichloropropane (D-D mixture). These are formulated with naphtha and other diluents, may contain emulsifiers, or be adsorbed on clay for application in granular form. These are often used in fields which are planted to vegetables, pineapples, or other edible crop plants.

Normal application rates for mineral soils range from 54 to 108 lbs. per acre for EDB, 17.6 to 88 lbs. for DBCP, and 100 to 200 lbs. for technical dichloropropene or 200 to 400 lbs. for D-D mixture.

These materials are most efficiently applied by injection into the soil at a depth of 6 to 8 inches in rows 12 inches apart over the whole field. An alternative method is to apply the nematocide to only the planting rows and leave the middles untreated. Since these materials are fumigants, the fumes diffuse in all directions from the application rows and kill nematodes in the upper 18 inches of soil.

All these nematocides are more or less toxic to plants when applied to the soil, but after a period this effect is no longer noticeable, depending on the chemical, the crop plant, and the soil type. EDB and dichloropropene are toxic to most kinds of plants for several days or two weeks after application. Some crop plants such as cotton are not injured by DBCP and can be planted immediately after application. On the other hand, tobacco might show injury from DBCP even if planted several months after application.

Ethylene dibromide leaves in the soil a residue containing bromine, which is taken up by various plants. In one experiment, tobacco and tomato plants grown in soil fumigated with EDB contained 4 to 50 times as much bromine as plants grown on the same soil not fumigated. The lower concentration was found in plants grown in loamy sand and the higher rate in plants grown in clay. Drying of the soil after fumigation affected the bromine content of the plants very little. This was interpreted as indicating that the bromine was present in inorganic form (Brown, et al., 1958). Another report indicates that the bromine is in the sorbed phase, the sorption being closely related to the surface area of the soil and also to the organic matter, moisture and clay contents (Call and Hague, 1957).

In an experiment in Virginia, peanut hay from plots treated with EDB contained 243 to 352 p.p.m. of bromine as compared with 25 to 60 p.p.m. in hay from adjacent untreated plots. When the hay from the treated plots was fed to cows for 4 weeks, there was a rise to a maximum of 109 p.p.m. of bromine in the milk (Young et al., 1955).

[1]Nematologist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture.

A number of tolerances for bromine in vegetables and fruits grown in soil fumigated with EDB have been set by the Food and Drug Administration. These range from 5 parts per million of inorganic bromide calculated as bromine in lima beans and strawberries to 75 p.p.m. in potatoes, melons, parsnips, and carrots.

Information on residues from DBCP is not available, but it is apparent that some bromine remains in the soil. This fact is shown by bromine toxicity symptoms on plants especially sensitive to bromine, such as onion seedlings grown in treated soil (O'Bannon, 1958).

Dichloropropenes and D-D mixture hydrolyze and form hydrochloric acid in the soil (Shaw, 1948), but other breakdown products or residues in soil or plants under normal conditions of use have not been reported. However, it is quite certain that plants can take up something derived from these materials since toxicity symptoms appear if various kinds of plants are planted too soon after application. There are also reports of flavor tainting of vegetable grown in soil treated with excessive amounts.

Technical dichloropropenes have been approved for preplanting fumigation of soil to be used for 20 kinds of vegetables and 7 kinds of fruits on a nonresidue basis.

In addition to the materials already mentioned, 5 nematocides are used mostly on seedbeds, nurseries, greenhouses, or other small plots of valuable soil. These include methyl bromide, chloropicrin, sodium methyldithiocarbamate (SMDC), O,2-dichlorophenyl O,O-diethyl phosphorothioate (VC-13), and 3,5-dimethyltetrahydro-1,3,5,2H-thiadiazine-2-thione (DMTT). All these materials combine nematode control with other features, such as control of soil insects, soil fungi, and weed seeds. For convenience, these are called seedbed nematocides to differentiate them from the field nematocides discussed previously.

Of the seedbed nematocides, methyl bromide is most widely used, particularly on tobacco seedbeds. Since it is an odorless gas at temperatures above 38° F., it is usually formulated with 2 percent chloropicrin, which serves as a warning agent. Other formulations contain xylene as a solvent and can be handled as liquids at ordinary temperatures. Mixtures of methyl bromide and other chemicals are being tested experimentally. Methyl bromide is regularly used at rates of 1 to 2 lbs. per 100 square feet and is applied under a plastic cover, which is left in place 24 to 48 hours. Soil fumigated with methyl bromide has a residue containing bromine. This is sometimes troublesome with plants easily injured by bromine. However, most crop plants can be planted without injury in methyl bromide treated soil 72 hours after the cover has been removed. Methyl bromide is registered on a nonresidue basis for preplant soil treatment at 2 lbs. per 100 square feet with an aeration period of 72 hours.

Chloropicrin is mostly used in greenhouses and on seedbeds at application rates ranging from 200 to 1,000 lbs. per acre. Application is by injection into the soil at 10- or 12-inch intervals. In the soil chloropicrin is adsorbed in amounts directly correlated with the percentage of clay. Organic matter adsorbs little chloropicrin (Stark, 1948).

Aside from the well-known fact that chloropicrin is highly toxic to plants, both in soil and air, very little is known about its uptake by plants or the occurrence of residues derived from it in plants. It is registered on a non-residue basis for use on soil to be planted to strawberries or for vegetable seedbeds. Application of 482 lbs. per acre is permitted 2 weeks before planting. For use on celery seedbeds it is registered on a non-residue basis for use at 958 lbs. per acre 2 weeks before planting.

Sodium methyl dithiocarbamate (SMDC) is applied in water solution at rates of 1/2 to 1 lb. per 100 square feet and is washed down into the soil by liberal applications of water. It decomposes in the soil and some of the decomposition products have a fumigant

action. Other breakdown products remain in the soil and are toxic to both weeds and crop plants for a week or more after application. SMDC is registered on a non-residue basis for any crop if used at 490 lbs. per acre 7 days before planting on light soils, or 14 days before planting on heavy soils.

VC-13, a phosphorothioate compound, is reported to have a long residual action. This fact implies excellent stability in the soil. Residues have been found in various edible parts of crop plants by measuring the cholinesterase inhibition. The largest residues were found in root crops, much smaller ones in leaves, and only insignificant residues in true fruits (Boyd, 1959). VC-13 is registered for use on a non-residue basis; the application rate being 150 gallons of the 75 percent solution per acre prior to planting corn, cucumbers, peppers, squash, strawberries, or tomatoes.

The application rate for DMTT is 1/2 to 1 lb. per 100 square feet. In the soil, it is reported to hydrolyze soon after application. Breakdown products are methyl isothiocyanate, formaldehyde, hydrogen sulfide, and monomethylamine, but it is believed that these degrade further (Torgeson, et al., 1957). DMTT is approved for use on a non-residue basis for treatment of soil for cabbage, peppers, and tomatoes at 275 lbs. per acre with an aeration period of 3 weeks.

It is evident that the available information on the fate of nematocides in plants and soils is meager and incomplete. Research on this subject should start with determination of the breakdown products of nematocides in soil and their persistence under various conditions. The next step is a study of the uptake of the nematocides and their breakdown products by crop plants and the location of these in the edible or other parts of the plants.

LITERATURE CITED

Boyd, G. R. 1959. Determination of residues of O,2,4-dichlorophenyl O,O-diethyl phosphorothioate (VC-13 Nematocide) by cholinesterase inhibition. Jour. Agr. and Food Chem. 7:615-617.

Brown, A. L., J. J. Jurinak, and P. E. Martin. 1958. Relation of soil properties to Br uptake by plants following soil fumigation with ethylene dibromide. Soil Sci. 86:136-139.

Call, F., and N. G. Hague. 1957. Control of plant nematodes. Reports on the Progress of Appl. Chem. 42:605-612.

O'Bannon, J. H. 1958. Application of emulsifiable dibromochloropropane in irrigation water as a preplanting soil treatment. U.S. Plant Dis. Rptr. 42:857-860.

Shaw, E. 1948. The effect of a soil sterilant (dichloropropene-dichloro-propane mixture) on the chemical, physical and microbiological properties of desert soils. Doctor's thesis, University of Arizona. 118 pp.

Stark, F. L. 1948. Investigations of chloropicrin as a soil fumigant. Cornell Univ. Agr. Exp. Sta. Memoir 278. 61 pp.

Torgeson, D. C., D. M. Yoder, and J. B. Johnson. 1957. Biological activity of Mylone breakdown products. Phytopath. 47:536. (Abs.)

Young, R. W., L. I. Miller, W. A. Hardison, and R. W. Engel. 1955. Bromide content of cows milk when fed forage grown on ethylene dibromide-treated soil. Dairy Sci. 38:619.

HERBICIDES IN PLANTS

W. C. Shaw, J. L. Hilton, D. E. Moreland, and L. L. Jansen[1]

The native vegetation in most geographical areas in the United States was not very efficient or economical as a source of food for livestock and humans. As the human population has increased, it has been necessary to replace the native vegetation with more productive, more efficient, and more economical plants for food and fiber (figure 1).

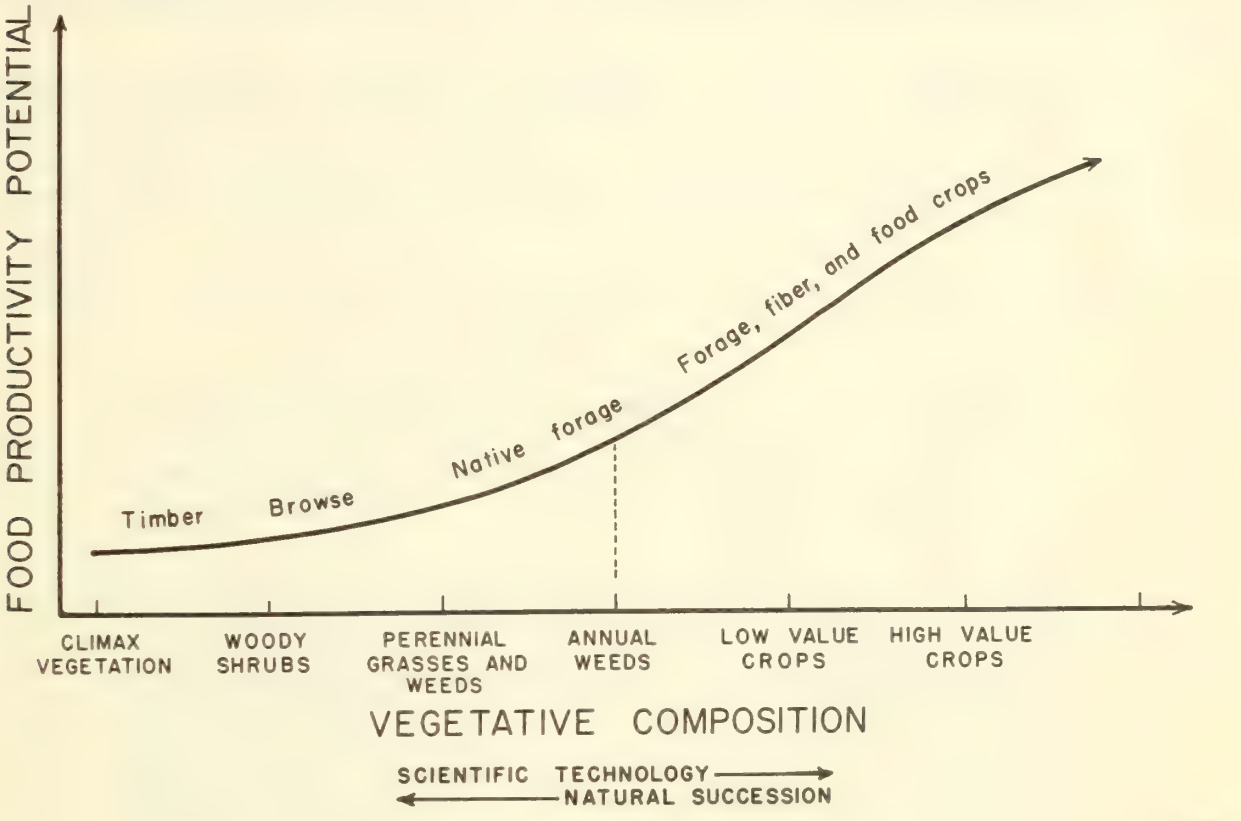


Figure 1

The sequence of events that occurs in plant successions when native vegetation is disturbed or cultivated fields are abandoned is well known. Weeds comprise an early stage of plant successions that terminate with the climax-vegetation characteristic of the area.

One of the basic objectives of agricultural research is to develop more efficient plants and soils and to manage them at high productivity levels. The fundamentals of plant ecology emphasize that plant successions always occur in the direction of the climax-vegetation rather than toward the growth of more productive economic crop plants.

In our efforts to produce economic crops, we attempt to utilize fully all available scientific technology to stabilize the vegetation at a highly productive level. The control of weeds is a basic, essential, and important aspect of this fundamental ecological process (figure 1).

---

[1]Plant Physiologists, respectively, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, at Beltsville, Md., and Raleigh, N. C.

Cultural, mechanical, biological, and chemical methods of weed control have been used for centuries. In recent years, herbicides have demonstrated outstanding potential for the selective control of weeds in crop production. If maximum benefits are to be realized from this vast, versatile, efficient, and economical source of energy for the control of weeds, we must develop a fundamental knowledge of the effects of herbicides on plants, soils, animals, and humans. The fate of herbicides in plants then becomes a critically important aspect of chemical weed control. It is an additional dimension in weed control research which must be better understood if we are to realize maximum benefits from the use of herbicidal chemicals.

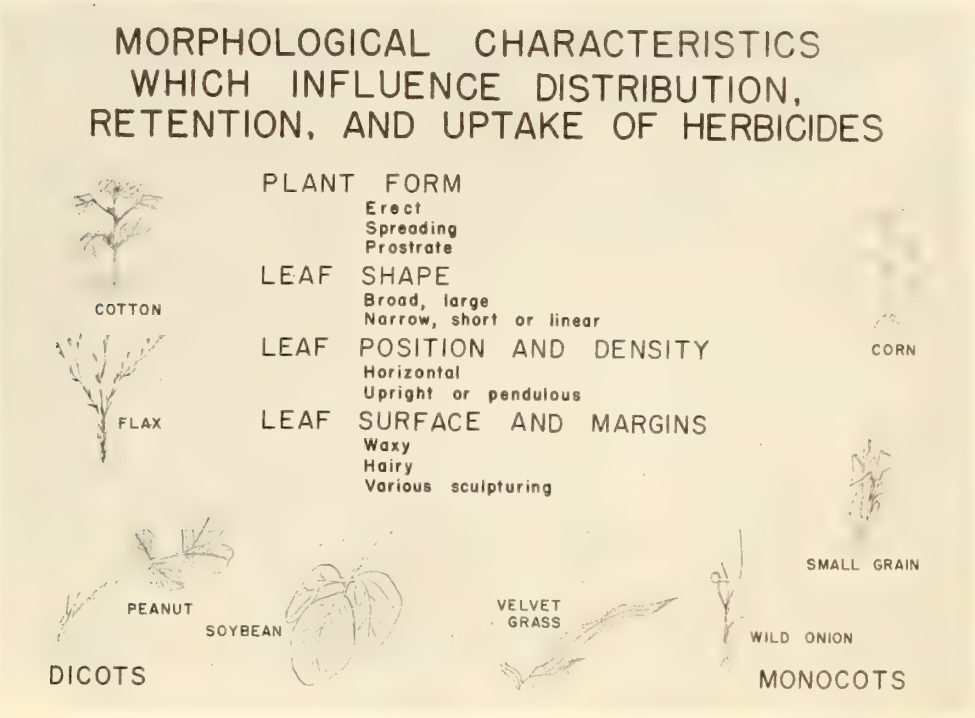
The purpose of this paper is to review some of the progress that has been made in determining the fate of herbicides in plants and to indicate some of the problems needing additional emphasis for maximum, efficient, and safe use of herbicides for weed control in crops.

In the time allotted it would be impossible to discuss all the research that has been reported in the literature on the fate of herbicides in plants. For this reason, we have chosen a series of slides to illustrate some of the progress that has been made and to discuss certain principles that have been developed from research on the penetration, absorption, translocation, mechanisms and sites of action, and metabolism of herbicides in plants.

The fate of herbicides in plants is determined by morphological characteristics, physiological processes, and biochemical properties of the protoplasm of plants as influenced by environmental factors that affect growth.

MORPHOLOGICAL CHARACTERISTICS WHICH INFLUENCE DISTRIBUTION, RETENTION, AND UPTAKE OF HERBICIDES

Plant form, leaf shape, leaf position, leaf density, leaf surface, and leaf margins greatly influence the foliar distribution, retention, and uptake of herbicides (figure 2).



BN-10802-X

Figure 2

Plants that have erect, spreading, or prostrate habits of growth possess different herbicide-recipient characteristics and retention-capacity patterns. Broad, large, narrow, short, or linear leaf shapes greatly influence herbicide reception, distribution, and retention patterns. Plants with upright, horizontal, or pendulous leaves which form foliage canopies of various densities differ markedly in retention and uptake of herbicides.

The nature of the leaf surface is critically important. Leaves with waxy, hairy or variously sculptured leaf surfaces differentially retain and absorb herbicides. Stomate distribution and nature of the cuticle probably determine the quantity of material that penetrates the outer layers of the leaf. The permeability barriers of the cell membranes may further decrease the amount of chemical absorbed into the individual cells.

The form, shape, size, density, distribution, and other characteristics of roots in relation to soil-applied herbicides also partly determine the amount of herbicide that actually comes in contact with the plant.

Collectively, the morphological characteristics of plants act as "obstacles" through which the herbicide must pass before the selective control of plant growth is achieved.

TRANSLOCATION OF HERBICIDES IN PLANTS

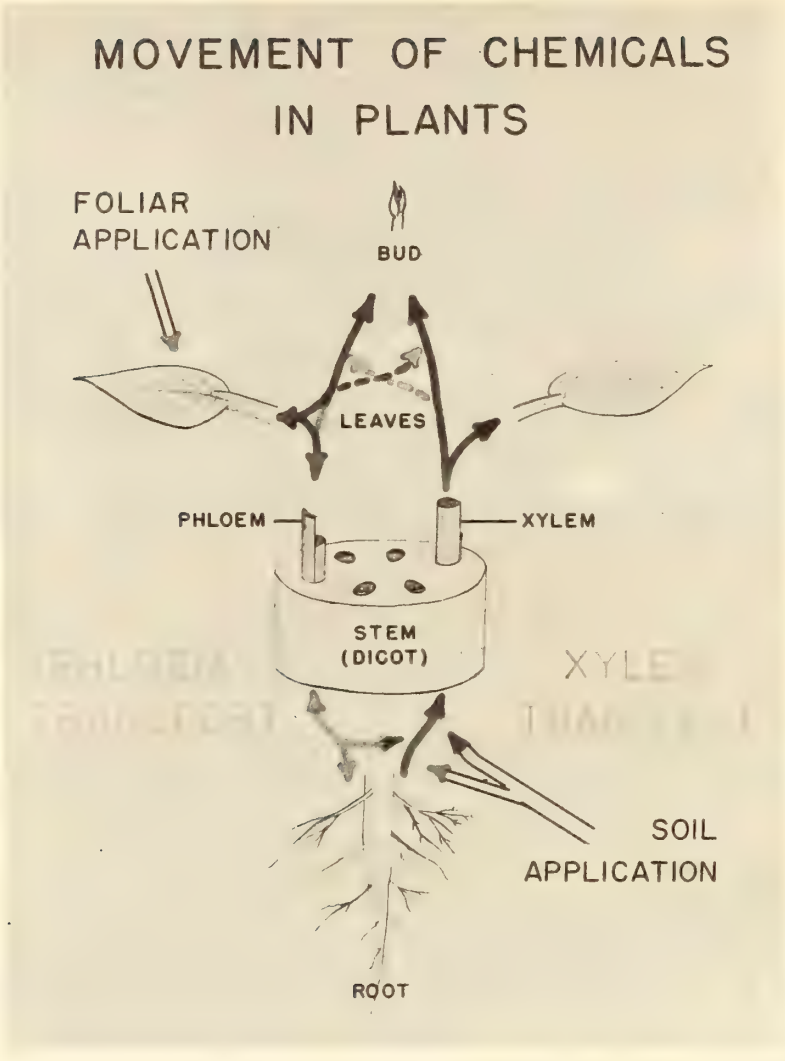
A voluminous literature exists on the translocation of herbicides in plants. No attempt has been made to review the significant results of these studies. Certain principles on the movement of herbicides have been established (figure 3).

Numerous factors influence the translocation of herbicides. Once the herbicide has been absorbed into the plant it may be immobilized in the treated tissue by adsorption to, or conjugation with, cell constituents. When some herbicides are absorbed the molecule is probably metabolized prior to translocation. Since the downward movement of foliar-applied herbicides occurs primarily in the phloem (living tissue) and the upward movement of soil-applied herbicides takes place mainly in the xylem (nonliving tissue), all factors which influence plant growth and the transport of inorganic and organic solutes, water, and mineral elements also affect the movement of herbicides in plants.

Herbicides are differentially translocated and the efficiency with which different plant species translocate herbicides varies greatly. For example, the intercalary meristem of certain grasses restricts the translocation of foliar-applied herbicides. Certain translocation barriers also exist in perennial herbaceous and woody plants.

There is increasing evidence that lateral movement of herbicides between the phloem and xylem occurs. The mechanisms, energy concepts, and interrelationships of the translocation of herbicides in plants are not sufficiently understood for their most effective use.

Our present knowledge of the translocation of herbicides, nevertheless, indicates that there are "translocation obstacles" which prevent herbicides from reaching their site(s) of action in the cell in sufficient quantity to control plant growth. These same obstacles also influence the differential selectivity of herbicides.



BN-10801-X

Figure 3

MECHANISMS AND SITES OF HERBICIDAL ACTION

Herbicides modify and control plant growth by inhibiting essential physiological processes or by preventing the biosynthesis of metabolites essential to plant growth (figure 4).

Figure 4 represents an over-simplified version of cellular metabolism. Carbohydrates, organic acids, and other products are synthesized through the process of photosynthesis. This food supply may be utilized in respiration to form the high energy phosphate bonds of adenosine triphosphate. This energy is then utilized in the biosynthesis of many cell constituents. The carbon skeletons synthesized through photosynthesis enter into enzyme-catalyzed reactions which lead in step-wise fashion to the production of growth-essential metabolites. These compounds are then incorporated into macromolecules such as coenzymes, proteins, nucleic acids, and polysaccharides. Macromolecules may then be incorporated into highly organized structures such as microsomes, mitochondria, spindle fibers, chloroplasts, and nucleus.

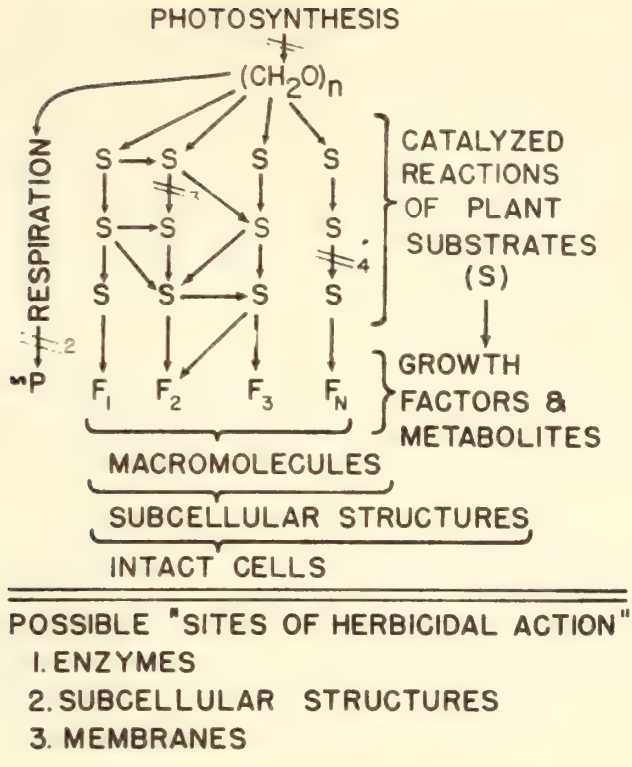


Figure 4

Some herbicides are known to act by inhibiting photosynthesis (position 1, figure 4) and essentially causing the cell to starve to death. Herbicides may also uncouple oxidative phosphorylation (position 2) and thereby eliminate many of the biosynthetic reactions of the cell. Many herbicides inhibit enzymes catalyzing specific reactions. If this inhibition occurs in a metabolic pathway, illustrated by the block at position 3, the plant may not be damaged, for growth factor 2 can still be synthesized through other pathways. However, if the metabolic block occurs in position 4, then growth factor F_n becomes growth limiting.

The growth-controlling action of herbicides is not necessarily of a biochemical nature. Physical or chemical effects on cell membranes, spindle fibers, or other subcellular structures may disrupt the normal physiological processes of these highly organized cellular components. Most herbicides are known to inhibit many biochemical or physiological processes. Control of growth probably results from the cumulative effects of these many inhibitions.

No attempt will be made to review the results of investigations on the mechanisms and sites of action of herbicides but a few examples will be discussed to illustrate the interrelationships with the metabolism of herbicides.

The results of recent investigations on mechanisms of action of herbicides may illustrate the importance of basic investigations. In 1947, it was demonstrated that nonphytotoxic chemicals could be metabolized within plants to phytotoxic compounds (58). This important scientific breakthrough resulted in new concepts on the selective action of herbicides (62). Fundamental studies in 1954 showed that certain plants can metabolize the herbicidally inactive 4-(2,4-dichlorophenoxy)butyric acid [4-(2,4-DB)] through the process of beta-oxidation to the herbicidally active 2,4-dichlorophenoxyacetic acid [2,4-D]. Therefore, weeds and crop plants capable of beta-oxidizing 4-(2,4-DB) to 2,4-D will be killed by 2,4-D, while plants not capable of beta-oxidizing 4-(2,4-DB) are tolerant to

the inactive 4-(2,4-DB) and are not killed (62). The highly selective heribicide 4-(2,4-DB) was developed directly as a result of these investigations. This herbicide has shown excellent promise for the post-emergence control of broadleaved weeds in (a) cereals underseeded with certain forage legumes; (b) the establishment of pure stands of forage legumes; (c) forage legume seed-production fields; (d) flax; (e) tolerant crop plants such as corn or rice grown in the vicinity of crops highly susceptible to the phenoxyacetic and alpha-phenoxypropionic acids, such as cotton and grapes; and (f) certain other weed-crop situations (50). The new chemical should prove valuable for the control of weeds in cereal crops underseeded with legumes. The herbicides available prior to the introduction of 4-(2,4-DB) could not be used safely for the control of weeds in cereal crops underseeded with alfalfa or other legumes in this important phase of the corn-wheat-alfalfa-alfalfa type rotation.

Recent studies have also shown that 2-chloro-4,6-bis(ethylamino)-s-triazine [simazine], a very promising pre-emergence herbicide for weed control in corn, when absorbed by the roots of the corn plant was converted to a nonphytotoxic form. Therefore, corn shows considerable tolerance to simazine. On the other hand, many important weeds do not effectively metabolize simazine to an inactive chemical. These weeds are highly susceptible to injury and are easily controlled by simazine. This is an excellent example of a herbicidally active chemical being metabolized in the plant to an inactive chemical. Thus, the metabolic fate of simazine is opposite to that of 4-(2,4-DB) when it is converted. Research scientists have also found recently that simazine inhibits the efficiency of photosynthesis in plants (43). It was theorized that if simazine inhibited the efficiency of photosynthesis in plants, this action should be reversible if simazine-treated plants were supplied with an available source of carbohydrate. By feeding glucose through the leaves of simazine-treated plants, it has been possible to protect plants against the phytotoxic effects of simazine (43). Similar results have been obtained with some of the phenylurea herbicides (29).

A third fundamental study indicates another breakthrough in weed control research. In a series of investigations involving the response of bacteria (Escherichia coli), yeast, germinating seedlings, and intact plants to 2,2-dichloropropionic acid [dalapon], it has been shown that dalapon and several other chloro-aliphatic acids inhibit the enzymatic synthesis of pantothenic acid, a B-vitamin essential to plant growth. Pantothenic acid is made by an enzyme which couples beta-alanine to pantoic acid. This enzyme has been isolated. The herbicides were shown to be competing with pantoic acid. The response of plants to dalapon may be due to the temporary inhibition of pantothenic acid synthesis. To examine this hypothesis, pantothenic acid was supplied to barley plants through their leaves. The exogenous source of pantothenic acid partly protected dalapon-treated plants against the herbicidal action of dalapon (34).

The N-phenylcarbamate herbicides at low concentrations stimulate respiration in root tips of treated plants while higher concentrations markedly decrease respiration (57). These herbicides are also potent inhibitors of chlorophyll synthesis and cause striking mutagenic effects in plants (51).

The phenoxyalkylcarboxylic acids may cause striking temporary changes in the chemical composition of plants (56). Numerous other instances of important breakthroughs resulting from fundamental research could be cited, but I believe these are adequate to indicate the critical need for additional fundamental research in weed control.

METABOLISM OF HERBICIDES

The various studies conducted during the last 10 years on the fate of herbicides in plants show that most herbicides are metabolized in varying degrees by plants. Some herbicides are metabolized so rapidly and so extensively that we are not certain of the toxic form of the chemical actually involved in the control of growth. Species may differ greatly in either (a) their ability to metabolize a specific herbicide and (b) the biochemical pathways by which the structure of the original molecule is altered. For some chemicals, these differences seem to be the determining factor in herbicidal selectivity.

Phenoxyalkylcarboxylic acids and derivatives

The phenoxyalkylcarboxylic acids are seldom applied as chlorinated acids. Application is usually made in the form of esters, organic amine salts, and other salts. Evidence from many sources suggests that conversion to the free acid is necessary for the expression of herbicidal activity. Esters of 2,4-D are hydrolyzed to the free acid by enzymes obtained from a wide variety of plant tissues (31,44). Evidence has been obtained in vivo to indicate that the isopropyl ester of 2,4-D is hydrolyzed in barley leaves and that the acid moiety of the original molecule is then translocated to other parts of the plant (11).

A new principle for the selective control of weeds in crops was established with the discovery that plant species differ in their capacity to β-oxidize the lengthy side chain of chloro-substituted phenoxyalkylcarboxylic acids. Certain ones of the long-chain chemicals are nonphytotoxic unless the plant can degrade the side chain to yield the corresponding acetic acid derivative. The literature pertaining to β-oxidation of chemicals in plants has been recently reviewed by Wain (61).

The L-amino acid derivatives of 2,4-D are more active against intact plants than the corresponding D-derivatives (39,49). Conversion to the free acid probably occurs by enzymatic hydrolysis of the peptide linkage in plants or possibly in microorganisms of the soil. Soil microorganisms are responsible for the activation of at least one nontoxic chemical. Plants are not killed by sodium 2,4-dichlorophenoxy-ethyl sulfate [sesone] applied to the foliage. However, this chemical is converted to 2,4-D in the soil and the toxic compound may then be absorbed through the roots (60). The amount of chemical available in toxic form for growth-controlling actions partly depends on the plant's ability to convert inactive chemicals to toxic ones.

A second obstacle keeping the herbicide from its site(s) of action may be absorption of the free acid at sites not involved in the growth response. Experiments with 2-methyl-4-chlorophenoxyacetic acid [MCPA] show that this compound is adsorbed to materials obtained from plant tissues (7,8). The resistance of plants to MCPA seemed to be correlated to their ability to absorb the chemical (7).

There is now considerable evidence that the 2,4-D molecule itself is metabolized by plants (2,9,15,16,18,32,35,36,37,38,64,65,66,67). Metabolism of 2,4-D includes (a) minor alterations in structure of the parent molecule (35,65), (b) partial degradation with loss of the acetic acid side chain (18,36,64,65,66,67), and (c) conjugation of 2,4-D or its metabolites with plant substrates (9,15,16,35,38). The quantity and identity of the complex 2,4-D conjugates formed by plants vary among species (9,15,16), but the selective action of the herbicide does not appear to be correlated with differences in the reaction of 2,4-D with plant constituents in susceptible and resistant plants (15). Corn and beans behave similarly in their ability to degrade 2,4-D. Both species slowly release the carbon atoms of the 2,4-D side chain as CO_2 (66). If degradation of the 2,4-D molecule represents a detoxification mechanism, it seems unlikely that this type of detoxification can be responsible for the selective action of 2,4-D. Plants have a limited capacity to produce CO_2 from 2,4-D, and the CO_2 released per day probably represents a very small percentage of the 2,4-D applied in the field. Plants can also alter the structure of 2,4-D without loss of the acetic side chain (35,65). Hydroxylation of the ring has been suggested as a possible process in formation of one of the non-2,4-D acids recovered from plant extracts (35). Evidence for ring hydroxylation has been obtained for certain phenoxy-aliphatic acids (68).

Investigations on the fate of 2,4-D in plants are in agreement that a relatively small percentage of the herbicide can be recovered from metabolically active plants as the unaltered molecule. It is not known whether 2,4-D or one of its metabolized forms is the toxic principle responsible for the major biological and biochemical changes observed in plants treated with 2,4-D. Consequently, the various types of metabolism of the herbicide in plants could represent either activation or detoxification mechanisms.

s-Triazine Herbicides

Degradation of C^{14}-ring-labeled simazine and 2-chloro-4-ethylamino-6-isopropylamino-s-triazine [atrazine] in the corn plant (a tolerant species) is indicated by the early appearance of $C^{14}O_2$ released to the atmosphere (24,41,42). Only traces, if any, of simazine or atrazine remained in the plant at time of harvest (41). Cell-free preparations from corn tissues release little or no CO_2 when incubated with simazine (21), but the herbicide molecule was altered (21,24,48). Hydroxy simazine appears to be the first metabolic product in the degradation of simazine (24,30). Cell-free preparations from wheat, a simazine-susceptible plant, did not alter the structure of the herbicide (48).

Studies with C^{14}-ring-labeled simazine show that both tolerant and susceptible species absorb the chemical. However, unaltered simazine either cannot be detected in the foliage of tolerant plants or is present only in very small amounts. The amount of nonmetabolized simazine, or biologically active C^{14}-labeled degradation products, which accumulates in oats, cucumber, cotton, and corn, is correlated with the relative susceptibility of the four species (13,52,53). Some degradation of the C^{14}-labeled herbicide is indicated in cotton and cucumber as well as in corn since $C^{14}O_2$ was released from all three species (14). Solvent extracts of oat and cotton tissues showed that measurable amounts of the absorbed simazine was metabolized. With intact plants most of the simazine which was metabolized appeared to be converted in the roots. Roots of the two species degraded approximately equal proportions of simazine. Differences in the rates of accumulation of the herbicide in leaves of the two species were closely correlated with differences in rates of transpiration (52,53).

Chloro-substituted aliphatic acid herbicides

Although there have been few published reports concerning the metabolic fate of the chloro-substituted aliphatic acids in plants, the available literature indicates that dalapon and trichloroacetic acid [TCA] are not readily metabolized in plants. No metabolic products of C^{14}-labeled TCA could be found in corn or peas four days after treatment (5). Similar experiments with corn and soybeans treated with C^{14}-labeled dalapon showed no evidence of metabolism of the herbicide. Four days after treatment, 99 percent of the applied radioactivity was recovered by extraction and identified as dalapon (6). Dalapon remains essentially nonmetabolized in plants for long periods, but some slow decomposition is indicated by the release of Cl^{36} (from dalapon-Cl^{36}) or the incorporation of C^{14} (from dalapon-2-C^{14}) into other compounds (21,22,23).

Substituted phenylurea herbicides

Ethanol extracts of leaves from bean plants treated with carbonyl-C^{14}-labeled 3-(p-chlorophenyl)-1,1-dimethylurea [monuron] contained two major radioactive compounds (17). One of the compounds was identified as unchanged monuron. The amount of monuron in the tissue decreased with time. The amount of the second compound increased. The unknown was considered to be a monuron complex since monuron was released by acid hydrolysis. Degradation of monuron in plants was suggested by the rapid reduction of radioactivity after the fourth day following treatment.

Carbamates

The lower limit of sensitivity of the analytical methods for isopropyl N-phenylcarbamate [IPC] and isopropyl N-(3-chlorophenyl) carbamate [CIPC] is 0.05 p.p.m. Analyses conducted on crops receiving high rates of chemicals have not shown residues which exceeded this amount (25,26,27). Apparently, these N-phenylcarbamates do not retain their identity in treated plants.

The failure to find unaltered IPC in treated plants stimulated a search for possible transformation products. Isopropyl N-hydroxy, N-phenylcarbamate (N-hydroxy-IPC)

possesses herbicidal properties similar to those of IPC. In contrast to IPC, N-hydroxy-IPC was active when applied to the foliage of oats. It has been suggested that IPC may be biologically oxidized within the plant to N-hydroxy-IPC. This was considered to be an activation reaction (4).

Ethyl N,N-di-n-propylthiolcarbamate [EPTC] is readily absorbed by plants from soil. Kidney beans, sweet corn, garden peas, radishes, carrots, cabbage, mustard, table beets, and sugar beets were treated with S^{35}-labeled EPTC. The largest amount of free EPTC-S^{35} remaining in the tissues several weeks after treatment was less than 3 percent of the total amount absorbed (19).

In relatively short-term experiments, the amount of EPTC-S^{35} decreased with time in resistant seedlings but no decrease was observed in susceptible species (20). Identification of radioactive compounds obtained from treated plants suggested that the sulfur atom was oxidized to sulfate. The sulfate was then incorporated into normal sulfur-containing metabolites including cysteic acid, cystine, methionine, methionine-sulfone, and two unidentified compounds.

Amitrole

Much of the information concerning the metabolism of 3-amino-1,2,4-triazole [amitrole] in plants is unpublished or available only in abstract form. Nevertheless, the published evidence indicates that amitrole is metabolized by plants (1,10,33,40,45,46,47, 69). The rate of metabolism and the biochemical reactions involved vary according to species (33,47,69). If any metabolized form of C^{14}-labeled amitrole is formed in corn, it is not accumulated by the plant. Soybeans, however, accumulate a C^{14}-labeled amitrole-metabolite at the expense of unaltered amitrole. Degradation of the herbicide in both species is suggested by the steady decrease in radioactivity that occurs during a 3-week period (69).

Various species of legumes rapidly metabolize amitrole. Only 7 percent of the radioactivity recovered from pinto beans 5 days after treatment with C^{14}-labeled amitrole was still in the form of the original molecule (45). As much as 2 mg. of metabolite per plant has been obtained from French dwarf bean 5 to 7 days after treatment (40). The amitrole-metabolites accumulated by various species of legumes seem to contain the same ring system and free amino group of the parent molecule plus an additional component (10,40,45). Radioactivity from C^{14}-labeled glycine or serine was incorporated into the amitrole-metabolite accumulated by Phaseolus vulgaris var. Black Valentine (10). The chemical properties of the major metabolite obtained from pinto beans suggested that the compound had an amino acid-like structure (45). The amitrole-metabolite from French dwarf bean was identified as 3-amino-1,2,4-triazolyl alanine. α-Alanine was obtained from this metabolite by chemical degradation (40).

Compound X and compound Y, the major and minor amitrole-metabolites recovered from pinto beans, were compared with amitrole for toxicity against duckweed (45). Compound X was not metabolized by duckweed. It caused some chlorosis and stunting but was not as toxic as the parent herbicide. Compound Y did not appear to be toxic. The formation of these compounds may, therefore, represent detoxification mechanisms which may or may not be a result of the toxic action of amitrole.

Amitrole reacts with glucose in vitro (28), and this compound is sometimes found in extracts from plant materials (33,46). The earliest report recognized that the compound might not be of metabolic origin (47). If the glucose complex is formed in amitrole-treated plants, it appears to be metabolized by the plant (55) and to serve as a substrate for hexokinase (28).

CDAA

Resistant crop seedlings such as soybeans and corn absorb 2-chloro-N,N,-diallyl-acetamide [CDAA] and completely metabolize it within 4 to 5 days after emerging from the soil. Glyoxylic acid is one of the earliest degradation products identified in the plant.

Conversion to glycolic acid is thought to occur rapidly. Susceptible species of plants are considered to lack the ability to metabolize CDAA (63).

Maleic hydrazide

1,2-Dihydro-pyridazine-2,6-dione (maleic hydrazide, MH) appears to remain intact in plants for long periods. The persistence of MH in tissue was recently reviewed and discussed by Smith, et al (54). MH is, however, known to react with certain plant constituents (59). Of the total radioactivity 15 percent obtained from tissue extracts of young wheat leaves incubated with C^{14}-MH was associated on paper chromatograms with spot U 1, approximately 1 percent with spot U 2, and the remainder was present as unaltered MH. Spot U 1 was identified as a β-glycoside of MH since it could be hydrolyzed with acid or the enzyme, emulsin, to yield glucose and MH. The authors (59) considered that the sugar moiety could be a disaccharide of glucose, for example, gentiobiose. No indication of the identity of spot U 2 was given. The formation of the glycoside could be a detoxification mechanism.

MH has also been shown to form complexes with other plant constituents (3). C^{14}-MH (carbonyl labeled) was shown to bind with proteins, such as egg albumin, zein, and hexokinase, and RNA in vitro. Stronger bonds were formed and more MH was bound when the MH was introduced as the diethanolamine salt rather than as the potassium salt. Fractions obtained by an ammonium sulfate precipitation of homogenates obtained from plants treated with C^{14}-MH were also found to contain labeled material. The radioactivity was not readily dissociated by overnight dialysis against either water or non-labeled MH. More radioactivity was present in fractions obtained from plants treated with the diethanolamine salt than from those treated with the potassium salt of MH. Forces involved in the binding action were considered to be physical rather than chemical.

DISCUSSION

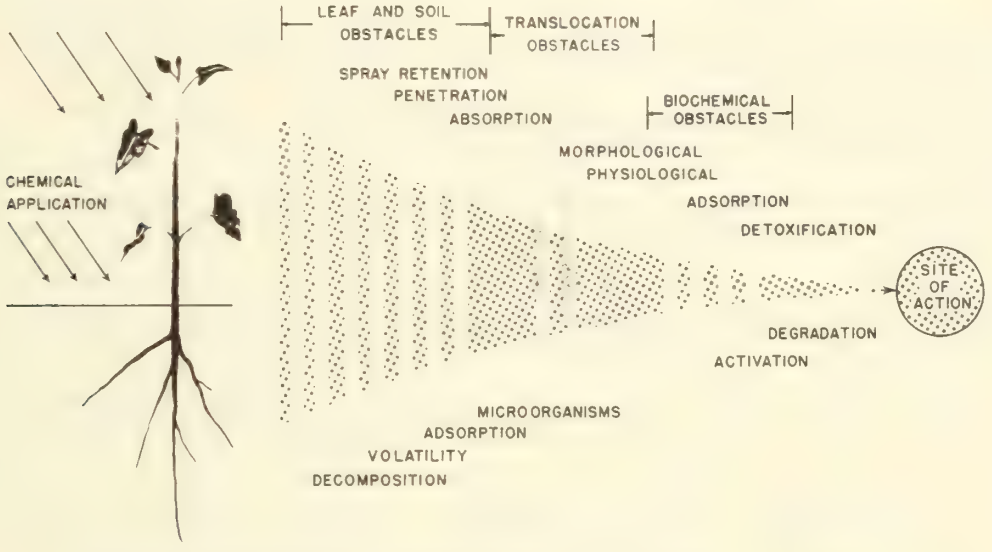
Any organic chemical introduced into a plant is subject to possible degradation, alteration of structure, or conjugation with natural plant constituents. There are, of course, differences in the ability of various species of plants to metabolize any individual herbicide. These differences, in some instances, provide the basis for their selective action on plants. The selective action of 4-(2,4-dichlorophenoxy) butyric acid is apparently the result of differences in the ability of different plants to convert the inactive chemical to the active acetic acid derivative. Future research may prove that other activation mechanisms are involved in the selective action of other herbicides.

Both resistant and susceptible species may be able to degrade herbicides such as simazine and probably others. The selective action of these chemicals may be due to differential absorption, translocation, and the rapidity with which various species accomplish detoxification through complete degradation. Detoxification does not necessarily depend on processes of degradation or even on metabolic reactions. The herbicides may be absorbed to cell constituents or enzymatically combined with plant substrates. Phytotoxic chemicals are known to be conjugated with proteins, carbohydrates, or amino acids. Formation of these complex conjugates may be a detoxification mechanism which completely inactivates the toxic material. In other instances, the herbicide-metabolite retains some of its toxic properties but is less toxic than the parent molecule.

Movement of the toxic material within the treated plant depends upon complex and interrelated morphological, physiological, biochemical, chemical, and physical factors. It has been suggested that certain herbicides do not move readily in plants unless metabolized to more translocable forms. Conjugation of herbicides with plant substrates may result in immobilization of the toxic material. Many herbicides seem to move primarily in association with carbohydrates, presumably in the phloem. Consequently, the general metabolic activity of the plant probably plays an important role in distribution of chemicals within the treated plants.

Most herbicides can be recovered from plant tissue as the original molecule even though a major percentage of the compound may have undergone a change in chemical structure. Consequently, we are not certain of the actual chemical structure through which many of the herbicides exert their growth-controlling actions. Conceivably, some herbicides are applied in one form, translocated in a degraded or metabolized form, and express inhibition at the site of action as still another chemical structure. Other chemicals seem to be absorbed, translocated, and accumulated at the site of action as the original molecule.

Whether herbicides arrive at the site(s) of action in plant cells as the originally applied molecule, or as a degraded toxic metabolite, the accumulative evidence available today suggests that morphological, soil, translocation, physiological, and biochemical "obstacles", and their interaction with environmental influences determine the selective action of herbicides by influencing the concentration of the herbicide that reaches the site of action at any one time (figure 5). It is also conceivable that the site(s) of action in different species possesses differential susceptibility to the toxicant.



OBSTACLES WHICH DETERMINE THE CONCENTRATION OF TOXIC MATERIAL AT THE SITE OF ACTION.

BN-10803-X

Figure 5

CONCLUSION

The fate of chemicals in plants is a relatively recent subject for scientific investigation. In view of the complexity of the subject, it is not surprising that after 10 years of study we still know comparatively little about the metabolism of herbicides in plants. Future research designed to uncover the principles which determine how and whether chemicals reach the growth site(s) involved in herbicidal action will undoubtedly aid greatly in the solution of the more difficult weed control problems.

If the vast, versatile, efficient, and economical source of energy in herbicide molecules is to be used effectively and safely to control weeds, fundamental research must be conducted to develop techniques to study the effects of herbicides and their breakdown products on plants, soils, animals, and man.

As new herbicides are developed their behavior in plants and their effects on animals, soils, and man must also be studied before safe, effective recommendations can be made to farmers.

LITERATURE CITED

1. Aldrich, F. D., and McLane, S. R. A paper chromatographic method for the detection of 3-amino-1,2,4-triazole in plant tissues. Plant Physiol. 32:153-154. 1957.

2. Ashton, F. M. Absorption and translocation of radioactive 2,4-D in sugar cane and bean plants. Weeds 6:257-262. 1958.

3. Baker, J. E. An investigation of the mechanism of maleic hydrazide in tobacco and other plants. Doctoral Dissertation, North Carolina State College, Raleigh, North Carolina. 1958.

4. Baskakov, Y. A., and Zemskaya, V. A. The possibility of transformation of carbanilic esters in plants. Fiziol. Rastenii Akad. Nauk S.S.S.R. [Transl.] 6:63-68. 1959.

5. Blanchard, F. A. Uptake, distribution and metabolism of carbon-14 labeled trichloroacetate in corn and pea plants. Weeds 3:274-278. 1954.

6. Blanchard, F. A., Muelder, W. W., and Smith, G. N. Herbicide uptake and distribution, synthesis of carbon-14 labeled dalapon and trial applications to soybean and corn plants. J. Agr. Food Chem. 8:124-128. 1960.

7. Brian, R. C. On the action of plant growth regulators. II. Absorption of MCPA to plant components. Plant Physiol. 33:431-439. 1958.

8. Brian, R. C., and Rideal, E. K. On the action of plant growth regulators. Biochim. et Biophys. Acta. 9:1-18. 1952.

9. Butts, J. S., and Fang, S. C. Tracer studies on the mechanism of action of hormonal herbicides. In A Conference on Radioactive Isotopes in Agriculture. Atomic Energy Commission Report Number TID-7512. pp. 209-214. 1956.

10. Carter, M. C., and Naylor, A. W. The formation in vivo of a complex between 3-amino-1,2,4-triazole and a glycine-serine derivative. Plant Physiol. 34 (sup.) vi. 1959.

11. Crafts, A. S. Evidence for hydrolysis of esters of 2,4-D during absorption by plants. Weeds 8:19-25. 1960.

12. Crafts, A. S., and Foy, C. L. Autoradiography of radioactive dalapon. Down to Earth 14(4):2-6. 1959.

13. Davis, D. E., Funderburk, H. H., Jr., and Sansing, N. G. The absorption and translocation of C^{14}-labeled simazine by corn, cotton, and cucumber. Weeds 7:300-309. 1959.

14. Davis, D. E., Funderburk, H. H., Jr., and Sansing, N. G. Absorption, translocation, degradation and volatilization of radioactive simazine. (Abs) Proc. Southern Weed Conf. 12th Meeting. pp. 172-173. 1959.

15. Fang, S. C. Absorption, translocation and metabolism of 2,4-D-C^{14} in pea and tomato plants. Weeds 6:179-186. 1958.

16. Fang, S. C., and Butts, J. S. Studies in plant metabolism. III. Absorption, translocation and metabolism of radioactive 2,4-D in corn and wheat plants. Plant Physiol. 29:56-60. 1954.

17. Fang, S. C., Freed, V. H., Johnson, R. H., and Coffee, D. R. Absorption, translocation, and metabolism of radioactive 3-(p-chlorophenyl)-1,1-dimethylurea (CMU) by bean plants. J. Agr. Food Chem. 3:400-402. 1955.

18. Fang, S. C., Jaworski, E. G., Logan, A. V., Freed, V. H., and Butts, J. S. The absorption of radioactive 2,4-dichlorophenoxy-acetic acid and the translocation of C^{14} by bean plants. Arch. Biochem. Biophys. 32:249-255. 1951.

19. Fang, S. C., and Theisen, P. An isotopic study of ethyl N,N-di-n-propylthiolcarbamate (EPTC-S^{35}) residue in various crops. J. Agr. Food Chem. 7:770-771. 1959.

20. Fang, S. C., and Yu, T. C. Absorption of EPTC-S^{35} by seeds and its metabolic rate during early stages of germination. Res. Prog. Rept., Western Weed Control Conf. pp. 91-92. 1959.

21. Foy, C. L. Personal communication.

22. Foy, C. L. The accumulation and persistence of dalapon in seeds of cotton and small grains. Res. Prog. Rept., Western Weed Control Conf. pp. 83-84. 1959.

23. Foy, C. L. Studies on the absorption, distribution, and metabolism of 2,2-dichloropropionic acid in relation to phytotoxicity. Doctoral Dissertation, University of California, Davis, California. 1958.

24. Freed, V. H. Personal communication.

25. Gard, L. N., Ferguson, C. E., Jr., and Reynolds, J. L. Effect of higher application rates on crop residues of isopropyl N-phanylcarbamate and isopropyl N-(3-chlorophenyl)carbamate. J. Agr. Food Chem. 7:335-338. 1959.

26. Gard, L. N., Pray, B. O., and Rudd, N. G. Residues in crops receiving pre-emergence treatment with isopropyl N-(3-chlorophenyl)carbamate. J. Agr. Food Chem. 2:1174-1176. 1954.

27. Gard, L. N., and Reynolds, J. L. Residues in crops treated with isopropyl N-(3-chlorophenyl)carbamate and isopropyl N-phenylcarbamate. J. Agr. Food Chem. 5:39-41. 1957.

28. Gentile, A. C., and Fredrick, J. F. Chemical activity of the glucose adduct of 3-amino-1,2,4-triazole. Physiol. Planatarum 12:862-867. 1959.

29. Gentner, W. A., and Hilton, J. L. Effect of sucrose on the toxicity of several phenylurea herbicides to barley. Weeds (In press).

30. Gysin, H., and Knusli, E. Chemistry and herbicidal properties of triazine derivatives. Processed publication, Geigy Agricultural Chemicals.

31. Hagen, C. E., Clagett, C. O., Helgeson, E. A. 2,4-Dichlorophenoxyacetic acid inhibition of castor bean lipase. Science 110:116-117. 1949.

32. Hay, J. R., and Thimann, K. V. The fate of 2,4-dichlorophenoxyacetic acid in bean seedlings. I. Recovery of 2,4-dichlorophenoxyacetic acid and its breakdown in the plant. Plant Physiol. 31:382-387. 1956.

33. Herrett, R. D. Studies on the absorption, translocation and metabolism of 3-amino-1,2,4-triazole in perennial plants. Dissertation Abstr. 20:2002. 1959.

34. Hilton, J. L., Ard, J. S., Jansen, L. L., and Gentner, W. A. The pantothenate-synthesizing enzyme, a metabolic site in the herbicidal action of chlorinated aliphatic acids. Weeds 7:381-396. 1959.

35. Holley, R. W. Studies of the fate of radioactive 2,4-dichlorophenoxyacetic acid in bean plants. II. A water soluble transformation product of 2,4-D. Arch. Biochem. Biophys. 35:171-175. 1952.

36. Holley, R. W., Boyle, F. P., and Hand, D. B. Studies on the fate of radioactive 2,4-dichlorophenoxyacetic acid in bean plants. Arch. Biochem. 27:143-152. 1950.

37. Jaworski, E. G., and Butts, J. S. Studies in plant metabolism. II. The metabolism of C^{14} labeled 2,4-dichlorophenoxyacetic acid in bean plants. Arch. Biochem. Biophys. 38:207-218. 1952.

38. Jaworski, E. G., Fang, S. C., and Freed, V. H. Studies in plant metabolism. V. The metabolism of radioactive 2,4-D in etiolated bean plants. Plant Physiol. 30:272-275. 1955.

39. Krewson, C. F., Drake, T. F., Neufeld, C. H. H., Fontain, T. D., Mitchell, J. W., and Preston, W. H. Amino acid derivatives of 4-chlorophenoxyacetic acid and their plant regulating effects in preliminary screening tests. J. Agr. Food Chem. 4:140-143. 1956.

40. Massini, P. Synthesis of 3-amino-1,2,4-triazolyl alanine from 3-amino-1,2,4-triazole in plants. Biochim. et Biophys. Acta. 36:548-549. 1959.

41. Montgomery, M., and Freed, V. H. The uptake and metabolism of simazine and atrazine by corn plants. Res. Prog. Rept. Western Weed Control Conf. pp. 93-94. 1959.

42. Montgomery, M., and Freed, V. H. The absorption, translocation, and metabolism of triazine herbicides by corn. Weed Soc. Amer., Proc. p. 41. 1960.

43. Moreland, D. E., Gentner, W. A., Hilton, J. L., and Hill, K. L. Studies on the mechanism of herbicidal action of 2-chloro-4,6-bis(ethylamino)-s-triazine. Plant Physiol. 34:432-435. 1959.

44. Morre, D. J., and Rogers, B. J. The fate of long chain esters of 2,4-D in plants. Weeds (In press).

45. Racusen, D. The metabolism and translocation of 3-aminotriazole in plants. Arch. Biochem. Biophys. 74:106-113. 1958.

46. Rogers, B. J. The action of 3-amino-1,2,4-triazole in plants. The Hormolog. 1:10-12. 1957.

47. Rogers, B. J. Translocation and fate of amino triazole in plants. Weeds 5:5-11. 1957.

48. Roth, M. W. Etude compareé de la réaction mais et du blea la simazin, substance herbicide. Compt. Rend. 245:942-944. 1957.

49. Shaw, W. C., and Gentner, W. A. Unpublished results.

50. Shaw, W. C., and Gentner, W. A. The selective herbicidal properties of several variously substituted phenoxyalkylcarboxylic acids. Weeds 5:75-92. 1957.

51. Shaw, W. C., and Swanson, C. R. The relation of structural configuration to the herbicidal properties and phytotoxicity of several carbamates and other chemicals. Weeds 2:43-65. 1953.

52. Sheets, T. J. The uptake, distribution, and phytotoxicity of 2-chloro-4,6-bis(ethylamino)-s-triazine. Doctoral Dissertation, University of California, Davis, California. 1959.

53. Sheets, T. J. The uptake and distribution of 2-chloro-4,6-bis(ethylamino)-s-triazine in oat and cotton seedlings. Weed Soc. Amer. Proc., pp. 44-45. 1960.

54. Smith, A. E., Zukel, J. W., Stone, G. M., and Riddell, J. A. Factors affecting the performance of maleic hydrazide. J. Agr. Food Chem. 7:341-344. 1959.

55. Sund, K. A. [Doctoral Dissertation cited by Gentile and Fredrick, reference number 28 above.]

56. Swanson, C. R., and Shaw, W. C. The effect of 2,4-dichlorophenoxyacetic acid on the hydrocyanic acid and nitrate content of Sudan grass. Jour. Amer. Soc. Agron. 46:418-421. 1954.

57. Swanson, C. R., Shaw, W. C., and Hughes, J. H. Some effects of isopropyl N-(3-chlorophenyl)carbamate and an alkanolamine salt of dinitro ortho secondary butyl phenol on germinating cotton seeds. Weeds 2:178-189. 1953.

58. Synerholm, M. E., and Zimmerman, P. W. Preparation of a series of omega-(2,4-dichlorophenoxy) aliphatic acids and some related compounds with a consideration of their biochemical role as plant growth regulators. Contr. Boyce Thompson Inst. 14:369-382. 1947.

59. Towers, G. H. N., Hutchinson, A., and Andreae, W. A. Formation of a glycoside of maleic hydrazide in plants. Nature 181:1535-1536. 1958.

60. Vlitos, A. J. Biological activation of sodium 2-(2,4-dichlorophenoxy)-ethyl sulfate. Contrib. Boyce Thompson Inst. 17:127-149. 1953.

61. Wain, R. L. The relation of chemical structure to activity for the 2,4-D type herbicide and plant growth regulator. Advances in Pest Control Research. II. pp. 263-305. 1958.

62. Wain, R. L., and Wightman, F. The growth-regulating activity of certain omega-substituted alkylcarboxylic acids in relation to their beta-oxidation within the plant. Roy. Soc. London, Proc., Ser. B. 142:525-536. 1954.

63. Wangerin, R. R. CDAA... Controls weed grasses in grass family crops. Farm Chem. 118(12):47-49. 1955.

64. Weintraub, R. L., Brown, J. W., Fields, M., and Rohan, J. Metabolism of 2,4-dichlorophenoxyacetic acid. I. $C^{14}O_2$ production by bean plants treated with labeled 2,4-dichlorophenoxyacetic acids. Plant Physiol. 27:293-301. 1952.

65. Weintraub, R. L., Reinhart, J. H., Scherff, R. A., and Schisler, L. C. Metabolism of 2,4-dichlorophenoxyacetic acid. III. Metabolism and persistence in dormant plant tissue. Plant Physiol. 29:303-305. 1954.

66. Weintraub, R. L., Reinhart, J. H., and Scherff, R. A. Role of entry, translocation and metabolism in specificity of 2,4-D and related compounds. In A Conference on Radioactive Isotopes in Agriculture. Atomic Energy Commission Report Number TID-7512. pp. 203-208. 1956.

67. Weintraub, R. L., Yeatman, J. N., Lockhart, J. A., Reinhart, J. H., and Fields, M. Metabolism of 2,4-dichlorophenoxyacetic acid. II. Metabolism of the side chain by plants. Arch. Biochem. Biophys. 40:277-285. 1952.

68. Willcox, M. Metabolism of phenoxyaliphatic acids in higher plants. Doctoral Dissertation, North Carolina State College, Raleigh, North Carolina. 1960.

69. Yost, J. F., and Williams, E. P. Some residue and metabolism findings in the development of 3-amino-1,2,4-triazole. Northeastern Weed Control Conf., 12th Meeting, Proc. pp. 9-15. 1958.

THE TOXICITY OF PESTICIDES FOR LIVESTOCK

R. D. Radeleff and R. C. Bushland[1]

INTRODUCTION

Our objective in the present discussion is to evaluate, in broad terms, the toxicities and hazards of modern pesticides for livestock, utilizing the accumulated evidence derived from our own researches and those of many other scientists. In this evaluation we believe we can show that the modern pesticides have been, and are being, used with complete safety to treated or exposed livestock and that such usage need not introduce hazardous residues in foods derived from livestock.

Pesticide development has been primarily in the field of organic chemistry, creating thousands of new compounds, but often utilizing those that had existed as chemical curiosities for a number of years.

Certain persons have repeatedly expressed doubts as to the adequacy of investigation that may have preceded the recommendation of these compounds for farm use. The word "adequate" has never been defined in this regard, but a general definition is "equal to a requirement or occasion; sufficient; suitable", with an alternate of "barely satisfactory; acceptable, but not remarkable." Within this definition we believe the investigations have been adequate.

Before a new pesticide may be registered by the U.S. Department of Agriculture for shipment in interstate commerce, evidence must be presented showing that it is effective for the proposed usage and is reasonably safe when so used. The required information may be supplied by any responsible individual or organization, including other USDA Agencies. State laws provide similar protection for products marketed within their borders.

Few chemicals can meet the dual requirements of effectiveness and safety. This is exemplified in that the ARS Entomology Research Division of USDA has studied more than 17,000 new chemicals as insecticides or repellants since 1947. About 3,000 of these were proposed and tested for use against livestock parasites. Because of ineffectiveness, toxicity hazard, residues in meat or milk, manufacturing difficulties, and other problems, most of the 3,000 were eliminated, leaving only 10 synthetic organic insecticides recommended by the Entomology Research Division for use on livestock during 1959.

This process of elimination functions in the investigation of all new pesticides, being carried out by industrial, commercial, private, and governmental research units.

The manner in which a chemical is used governs, to some degree, its probable toxicological hazard for livestock. Materials applied directly to the animals represent the greater danger for acute poisoning, while those applied to feed crops usually represent the greater danger for subacute or chronic poisoning.

While a modern pesticide may be put into interstate commerce on the basis of scientific data which indicate that it is reasonably safe and effective under prescribed conditions of use, misuse will inevitably occur. Some misuse is generally anticipated and considered in both the recommendations and the registration for use, but it is simply not possible to anticipate the extremes of misuse which certain farmers and ranchers can devise

[1] Veterinarian in Charge, Animal Disease and Parasite Research Division; and Investigations Leader, Livestock Insects Investigations, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Kerrville, Tex.

The vast majority of livestock losses due to pesticides have been the result of either deliberate misuse, failure to read the label, or careless exposure of dangerous chemicals where animals could drink or eat them.

Our discussion is dominated by insecticides because these materials are the most widely dispersed in usage, may be applied to animals, may be injected or otherwise introduced into them, may be put on the animals' feed as a part of normal plant protection, and may be left carelessly exposed. Herbicides represent a smaller part, because their use is generally limited to the destruction of undesirable plants in crops not grazed by livestock or pasture plants not normally eaten by livestock. Therefore, the opportunities for exposure of livestock are usually severely restricted.

Defoliants and fungicides are even more restricted in use.

Until 1940, compounds of arsenic, lead, lime, sulfur, and fluorine, almost all of an inorganic nature, were the prime insecticides. Arsenicals, copper compounds, oils, and chlorates dominated the field of herbicidal chemicals, again primarily as inorganic compounds. Mercurial and phenolic compounds dominated the fungicide field. Red squill, strychnine, phosphorus, and thallium compounds dominated the rodenticide field.

There was a sprinkling of products from plants. For fumigation, there were carbon disulfide, carbon tetrachloride, sulfur, and cyanides.

The metallic and phenolic compounds offered the greatest hazard to livestock. Arsenic and lead have been recognized for years by livestock authorities as being the leading causes of poisoning in livestock other than the naturally occurring elements, such as selenium, fluorine, and molybdenum.

THE MEANING OF TOXICITY AND HAZARD

Before going deeper into the problem of the toxicological hazard of pesticides to livestock, it is important that we understand the relationship of toxicity to hazard. It is axiomatic in the science of toxicology that virtually any chemical or physical entity will have harmful effects upon living organisms if applied or consumed in excessive amounts. Such daily accepted essentials as sunlight and rain are perfect examples; too much of either will destroy plant and animal life. A substance is more or less toxic according to the amount required to do damage. The most toxic substance we know is probably the botulinus toxin, while some of the least toxic ones are the staple foods we eat. A substance becomes a hazard if the normal use, or minor misuse of it, is likely to harm desirable organisms, regardless of the amount of the substance required compared to other toxic materials.

Because of this relationship, we often find that the less toxic of a pair of compounds is actually the more hazardous in use. We have given emphasis to this factor in relation to chemicals used in mosquito control (94), and Knipling has emphasized it for entomological practice in general (70). Let us, then, remember that toxicity can be measured for a compound in terms of grams or ounces, but the hazard of its use depends entirely upon the manner in which it is used.

There is a natural tendency to emphasize the relative toxicity of an insecticide rather than its relative hazard. A spray that is toxic at 0.1 percent appears to be more dangerous than one toxic at 1.0 percent. In practice, this must be correlated with the concentration usually employed. For example, if the more toxic compound is used at 0.025 percent and the less toxic at 0.5 percent, the less toxic compound represents the greater hazard because a twofold increase would produce poisoning, while a fourfold increase would be required for the more toxic material.

In the same manner, forage treated with 0.1 pound of insecticide producing toxicity in cattle at 10 mg./kg. is less hazardous than one applied at 4 pounds per acre with toxicity to cattle at 100 mg./kg.

Compounds capable of destroying one form of life must be suspected of being capable of at least injuring other forms. Stopping life processes, whether in an animal or a plant, involves the immobilization of essential enzymes or in some other way blocking the essential functions of individual cells. When the essential cells of the organism die or stop functioning, then so must the organism.

Life-destroying compounds are generally somewhat selective in action, permitting us, by intelligent choice of material, to destroy one form of life without harming those forms we consider desirable.

The classes of pesticides are not so well-defined as might be desired and their members could be discussed under several classes. Most herbicides have some bactericidal or fungicidal activity, although none are used for these purposes. Similarly, many fungicides are herbicidal, but we can select many that can be safely applied to plants. One of the first systemically active animal insecticides 2-pizalyl-1,3-indanedione (Pival) found its better place as a rodenticide. Several insecticides, such as Bayer 21/199, Bayer L 13/59, Ruelene, and ronnel have been shown to have an anthelmintic action (5,36,49,51,61,62,72,101).

Ultimately we must select those compounds which destroy these undesirable forms of life without creating a hazard to desirable plants, animals, or to humans.

To cite the specific data that have been obtained concerning the toxicity of pesticides by scientists of the Agricultural Research Service alone would require many hours; to include those from industry and the various state and commercial laboratories would require again as much time. Our references to published literature have been selected to establish principles rather than to provide a complete bibliography of this subject.

INSECTICIDES

The discovery of DDT as an insecticide by Müller in 1939 was a great advancement of our potential for insect control, and for it he received a Nobel prize. The low acute toxicity of DDT for most mammals suggested there would be no problems associated with its use. Several developments caused a revision of scientific attitudes toward the safety of DDT and toward several similar compounds then under development.

Appearance in Meat and Milk

Howell, et al, (64) found DDT in the milk of cattle sprayed with DDT. This was taken in many quarters to represent mechanical contamination of the udders by the insecticide, because at that time it was not an acceptable theory that such compounds could pass through the unbroken skin to the blood and be circulated. It was necessary for us, at Kerrville, to conduct similar studies and to devise closed systems for collection of milk from within the udder to prove beyond doubt that excretion was taking place. It was then argued that the cattle were licking themselves and absorbing the DDT from their digestive tracts. The authors and others at the Kerrville Laboratory then kept sprayed cattle in stanchions--muzzled except for a few minutes feeding time--and collected milk in sealed systems. Again, DDT was found in the milk. DDT was found in milk by a large number of workers (1,9,16,19,20,26,28,39,42,111,116,122).

From the beginning, none of us dealing with the toxicity of these compounds for livestock had any doubts concerning the speedy absorption of insecticides from any of the body surfaces, either external or internal. One does not doubt when animals are poisoned and die within 30 minutes of treatment without having licked themselves.

Although the evidence was overwhelming, even the Council on Pharmacy of the American Medical Association insisted that the tests must have been done by inept people and should be repeated by competent persons without delay. As we now realize, these early studies were sound and have stood the test of time.

During the same period, various scientists were appraising the effect of low level feeding of DDT. They determined that consistent effects upon rats could be produced with rather low levels of DDT in feed. Many workers, including those of USDA, ARS at Kerrville and Beltsville, established that DDT and other chlorinated hydrocarbons were stored in the fat and excreted in milk of treated livestock, and were not likely to be destroyed in cooking (21), whether exposure was by spraying or from ingestion, depending upon level of exposure, species, sex, and condition of the animals (3,7,9,10,14,15,16,18,19,22,25,26, 27,28,29,30,31,35,37,39,40,41,42,43,45,46,47,50,54,55,56,57,58,60,65,67,85,87,88,114,118).

As data and pressure accumulated against residues of chlorinated hydrocarbons in foods, it was more and more apparent that compounds that were not stored in tissues or excreted in milk were essential. Industrial and governmental laboratories fell to the task. Soon, safer compounds appeared, with virtually no problems of storage in animal tissues, (27), and occurring in extremely low amounts in milk (27,50), eliminating them from use, but promising the discovery of such materials that would not appear in milk.

There has been virtually no evidence to indicate that the residues of insecticides on feed and forage following recommended use have poisoned livestock. This is due, of course, to careful preliminary study and evaluation of the toxicological hazards and to proper recommendations by industry and government. Table 1 illustrates the levels of the compounds which have been fed to livestock for extended periods without evidence of poisoning.

While poisoning has not been observed in animals consuming insecticides as residue on feeds, the consumption has usually led to storage in their tissues or excretion in their milk, or both. The storage of the insecticides in tissues has never seemed to have a harmful effect upon livestock; rather, it is the amount of residue on the feed which produced the residue that determines the appearance of poisoning.

We may dispense with residues in animal tissues as being of no general significance to livestock health, and do so in clear conscience, but we are then confronted with their significance to people consuming animal products, for the level of residues in the tissues establishes the dietary level for people, and the level of intake for people is significant.

Our interest, then, in the appearance and disappearance of pesticide residues in tissues of livestock is one of concern for the welfare of the consumer. The significance of these residues in human foods has been and will be discussed by experts in that field.

As the analytical methods became more and more sensitive, experiments had to be repeated to prove or revise earlier conclusions concerning the amounts of residues present.

The analytical chemists were momentarily foiled by the animals in at least two cases. Aldrin was found to be converted by animals to its epoxide, dieldrin (6) and heptachlor to its epoxide (33,34,35,41). Older methods, specific for aldrin and for heptachlor, had shown absence of residues of these compounds, but their epoxides were shown to be present in appreciable quantities. Chlordane also was reported stored as a metabolite (32).

It is interesting to note that while hundreds of tolerances have been established for fruits, vegetables, and feeds for a number of insecticides, only four compounds have tolerances in meat, these being DDT, methoxychlor, toxaphene, and malathion. DDT may be present at 7 p.p.m. and methoxychlor at 3 p.p.m. in fat of cattle, sheep or hogs. Toxaphene may be present at 7 p.p.m. in fat of cattle, goats, and sheep. Malathion may be present at 4 p.p.m. in fat of cattle, hogs, and poultry. Insecticides may not be present in milk, although official tolerances of zero have been set only for malathion and methoxychlor. The tolerance is also zero for malathion in eggs.

These tolerances are indeed few compared to the total number of insecticides to which livestock are exposed.

TABLE 1.--Summary of results of feeding various levels of insecticides to cattle and sheep for various times, and the maximum fat residues resulting from such feeding. All feedings were harmless to the health of the animals.

Chemical	Animals	Levels fed in total diet	Period fed	Maximum fat content
		P.p.m.	*Weeks*	*P.p.m.*
Aldrin	Cattle	5	16	8
		10	16	49
		25	8	78
	Sheep	5	16	17
		10	16	55
		25	8	78
BHC, all isomers	Cattle	100	16	250
	Sheep	100	16	119
BHC, Lindane	Cattle	1	12	1.5
		10	12	8
		100	12	98
Chlordane	Cattle	10	16	13
	Sheep	10	16	16
	Cattle	25	8	18
	Sheep	25	8	12
DDT	Cattle	10	4	6.8
	Sheep	10	4	3.1
	Cattle	25	16	40
	Sheep	25	16	15
Dieldrin	Cattle	1	16	5.5
	Sheep	1	16	3.9
	Cattle	2.5	16	14.3
	Sheep	2.5	16	10.5
	Cattle	10	16	44
	Sheep	10	16	48
	Cattle	25	8	75
	Sheep	25	8	69
Endrin	Cattle	2.5	16	1.6
	Sheep	2.5	16	3.2
	Cattle	5.0	16	2.4
	Sheep	5.0	16	2.2
Heptachlor	Cattle	1.0	8	4
	Cattle	2.5	16	1.5
	Sheep	2.5	16	2.1
	Cattle	10	16	7.5
	Sheep	10	16	8.5
	Cattle	60	16	62
Methoxychlor	Cattle	25	16	0
	Sheep	25	16	0
Toxaphene	Cattle	10	8	less than 4
	Cattle	25	16	12
	Sheep	25	16	8
	Cattle	100	16	38
	Sheep	100	16	25

A manufacturer, in complying with regulations, may determine the maximum residues occurring in meat or milk after proposed use, show these quantities to be without hazard, and petition for a tolerance at that level. He might also establish the quantities remaining after a reasonable period of time, say 30 days, petition for a tolerance at that level, and then specify a 30-day delay between treatment and slaughter if the tolerance is granted. Alternatively, he can determine the time required for the residue to completely disappear, add a few days for safety, and make his recommendation include this time interval. When insecticides are not present, no tolerance is required.

Compounds that do not store and are not excreted in milk are, therefore, at a premium and would command much of the market if available. Very few can qualify without the protection of an interval of time between application and harvest or consumption.

The waiting period is not useful when producing dairy cattle are concerned. Since complete absence of residues in milk is the rule, direct application to the cattle is virtually ruled out for all except dusts of methoxychlor since the other materials available at this time are present in milk after treatment. The milk at that time must be destroyed or enter commerce illegally. Neither alternative is desirable. The waiting period can be used in feed or pasture work by keeping cattle off the crop or delaying harvest until the residues on the crop have disappeared.

Generally, these restrictions do not apply to dairy cattle not in production, but even in this case, some residues could appear at the onset of lactation if appreciable residues existed in the fat depots of the animals' body at that time.

Acute Toxicity

DDT, TDE, and methoxychlor were found to be virtually nontoxic for livestock and poultry when applied as sprays and of very low toxicity when given to them in feed or as medications (1,9,10,11,14,17,28,37,39,42,45,54,55,56,57,68,71,73,79,86,88,90,98,104, 113,115,120).

Chlordane, benzene hexachloride, toxaphene, strobane, dieldrin, aldrin, heptachlor, isodrin, endrin, and many other chlorinated hydrocarbons, and some organic phosphorus compounds were shown to be hazardous for livestock and poultry unless reasonable precautions were observed (2,4,8,15,17,24,36,44,49,62,66,69,74,78,83,84,86,89,90,96,97, 98,99,100,101,104,105,106,109,110,119,121). Early in our toxicological studies at Kerrville, we found that dairy calves 1 to 2 weeks of age were the most susceptible of all the farm animals, and that chickens were almost equally so. We also recognized that resistance to poisoning increased as the animals grew to maturity. Unfortunately, the passage of time brought chemicals showing equivalent toxicity for young and old animals. Summaries of the results of our toxicological studies are given in Tables 2 and 3.

We utilized this knowledge by selecting the young dairy calf as our standard test subject, representing the most susceptible species and age. The data derived from these studies formed a basis for safe recommendations for use of the chemicals. Although we desired the widest possible margin of safety between use dosage and minimum toxic doses, we decided that a twofold margin for young calves could be utilized on all other livestock if reasonable precautions were observed. (In other words, the recommended dose for livestock in general should not exceed one-half the minimum toxic dose for the young calves). After more than 12 years of using this criterion, we still feel confident of its usefulness, but have learned that there are exceptions and that factors other than dosage are equally important.

Effect of Formulation

Because most insecticides were not soluble in water, formulations were needed that would disperse or dilute them sufficiently for safe use. In general, simple solutions in

TABLE 2.--A summary of the minimum toxic and maximum non-toxic doses of insecticides administered orally to livestock at Kerrville, Tex.

Chemical	Animal	Age	Maximum non-toxic dose tested	Minimun toxic dose found
		CHLORINATED HYDROCARBONS		
			Mg./kg.	*Mg./kg.*
Aldrin	Calves	1-2 weeks	2.5	5.0
	Cattle	adult	10	25
	Sheep	adult	10	15
Benzene hexachloride as the gamma isomer	Calves	1-2 weeks	2.5	5
	Cattle	adult	10	25
	Sheep	adult	10	25
Chlordane	Calves	1-2 weeks	10	25
	Cattle	adult	75	---
	Sheep	adult	35	50
DDT	Calves	1-2 weeks	100	250
	Sheep	adult	250	500
TDE	Calves	1-2 weeks	100	250
	Sheep	adult	1000	---
Methoxychlor	Calves	1-2 weeks	250	500
	Sheep	adult	1000	---
Dieldrin	Calves	1-2 weeks	5	10
	Cattle	adult	10	25
	Sheep	adult	10	25
	Pigs	3 weeks	25	50
	Horses	adult	---	25
Heptachlor	Calves	1-2 weeks	15	25
	Sheep	adult	25	50
Heptachlor Epoxide	Calves	1-2 weeks	1.0	2.5
Toxaphene	Calves	1-2 weeks	2.5	5
	Cattle	adult	10	25
	Sheep	adult	10	25
	Goats	adult	---	50
		ORGANIC PHOSPHORUS COMPOUNDS		
Bayer L 13/59 (Dipterex)	Calves	1-2 weeks	5	10
	Cattle	adult	50	75
	Sheep	adult	200	---

TABLE 2.--A summary of the minimum toxic and maximum non-toxic doses of insecticides administered orally to livestock at Kerrville, Tex.--Continued

Chemical	Animal	Age	Maximum non-toxic dose tested	Minimum toxic dose found
		ORGANIC PHOSPHORUS COMPOUNDS--Continued		
			Mg./kg.	*Mg./kg.*
Bayer 21/199 (Co-Ral)	Cattle	adult	25	50
	Sheep	adult	15	25
Ronnel (Trolene)	Calves	1-2 weeks	100	125
	Cattle	adult	100	125
	Sheep	adult	400	250
4-tert-butyl-2-chlorophenyl methyl methylphosphoramidate (Ruelene)	Calves	1-2 weeks	25	50
	Sheep	adult	150	200
	Goats	adult	150	200
	Horses	adult	25	50
Bayer 17,147 (Guthion)	Calves	1-2 weeks	0.1	0.5
	Sheep	adult	12.5	25
Delnav	Calves	1-2 weeks	---	5
Diazinon	Calves	1-2 weeks	0.5	1.0
	Cattle	adult	10	25
	Sheep	adult	20	30
Malathion	Calves	1-2 weeks	10	20
	Cattle	adult	50	100
	Sheep	adult	50	100

oils or organic solvents were not desirable, because of the possibility of increased absorption, or the messiness which follows the oiling of an animal's hair. Three basic types of formulations were developed for marketing to livestock owners; dusts, wettable (suspensible) powders, and emulsifiable concentrates.

Dusts have offered fewer problems than the others, but have had limited usage because they were, in general, not retained by the treated animals as long as spray deposits. Nevertheless, they have had success in treating poultry, sheep, and to some degree other animals, particularly when treatments were required in cold weather.

Dusts are less likely to be absorbed by treated animals, concentration for concentration, than emulsions or wettable powders. This advantage is generally lost because of the greater concentration required for insect control (82).

Wettable or suspensible powders have given trouble, usually because their specific gravity is relatively high, allowing the particles to drop rapidly to the bottom of containers. This has a protectant effect if the powders are used in dipping vats (baths) since the effect is to decrease the concentration of the supernatant fluid--undesirable from the standpoint of parasite control. When used in spraying devices, the settling can be extremely dangerous, depending upon the mechanical design of the equipment. Without adequate agitation, and with the outlet of the tank at the bottom, the concentration in the fluid is first increased, dangerous to animals, then decreased, reducing parasite control. The reverse is true with suction applied from the top of the tank downward.

We also learned that the use of "stickers" (chemicals causing insecticides to adhere to treated surfaces) and suspending agents could significantly increase the amounts of

TABLE 3.--A summary of the minimum toxic and maximum non-toxic doses of insecticides applied as sprays or dips to livestock as determined at Kerrville, Tex.

Chemical	Animal	Age	Maximum non-toxic dose tested	Minimum toxic dose found
		CHLORINATED HYDROCARBONS*		
			Percent	*Percent*
Aldrin	Calves	1-2 weeks	0.1	0.25
	Lambs	3 weeks	---	4.0
	Kids	3 weeks	---	4.0
Benzene hexachloride, expressed as gamma isomer (Normal animals, not emaciated or lactating)	Calves	1-2 weeks	.025	.05
	Calves	6-8 months	.1	---
	Cattle	adult	.1	.25
	Lambs	3 weeks	1.0	---
	Sheep	adult	1.0	---
	Pigs	3 months	1.0	---
	Horses	adult	.15	---
Chlordane (Normal animals)	Calves	1-2 weeks	1.0	2.0
	Cattle	adult	2.0	---
	Lambs	3 weeks	1.0	2.0
	Sheep	adult	3.0	4.0
	Goats	adult	3.0	4.0
	Horses	adult	1.5	---
Dieldrin	Calves	1-2 weeks	.1	.25
	Cattle	adult	1.0	2.0
	Lambs	2 weeks	2.0	3.0
	Sheep	adult	---	4.0
	Goats	adult	---	4.0
	Pigs	8 weeks	4.0	---
	Horses	adult	1.0	---
Heptachlor	Calves	1-2 weeks	.25	.5
	Cattle	adult	.5	---
	Lambs	3 weeks	4.0	---
Toxaphene	Calves	1-2 weeks	.5	.75
	Cattle	adult	2.0	4.0
	Lambs	6 weeks	4.0	---
	Sheep	adult	1.5	4.0
	Kids	6 weeks	4.0	---
	Goats	adult	1.5	4.0
	Hogs	adult	4.0	---
	Horses	adult	1.5	---
		ORGANIC PHOSPHORUS COMPOUNDS		
Bayer 21/199(Co-Ral)	Calves	1-2 weeks	.25	.5
	Cattle	adult	1.0	2.0
	Sheep	adult	.25	.5
	Goats	adult	.25	.5
	Horses	adult	1.0	---
Diazinon	Calves	1-2 weeks	.05	.1
	Cattle	adult	.1	---

TABLE 3--A summary of the minimum toxic and maximum non-toxic doses of insecticides applied as sprays or dips to livestock as determined at Kerrville, Tex.--Continued

Chemical	Animal	Age	Maximum non-toxic dose tested	Minimum toxic dose found
		ORGANIC PHOSPHORUS COMPOUNDS--Continued		
			Percent	*Percent*
Malathion	Calves	1-2 weeks	0.5	1.0
	Cattle	adult	2.0	---
	Sheep	adult	1.0	---
	Goats	adult	1.0	---
Parathion	Calves	1-2 weeks	---	.01
	Sheep	adult	---	1.0
	Goats	adult	.1	1.0
Ronnel	Calves	1-2 weeks	2.5	---
(Dow ET-57, Korlan)	Cattle	adult	2.5	---
	Sheep	adult	2.5	---
Bayer L 13/59	Calves	1-2 weeks	1.0	---
(Dipterex)	Cattle	adult	2.0	---
4-tert-butyl-2-chlorophenyl	Calves	1-2 weeks	2.0	---
methyl methylphosphoramidate	Cattle	adult	.5	---
(Ruelene)	Sheep	adult	.5	---
Delnav	Calves	1-2 weeks	.25	.5
	Cattle	adult	.5	---
		CARBAMATES		
Sevin	Calves	1-2 weeks	2.0	4.0
	Cattle	adult	4.0	---

*DDT, TDE, Methoxychlor, Perthane, and Dilan were nontoxic to 1- to 2-week old calves at 8 percent concentrations.

insecticide left on hair, and that this effect was altered by the degree of hardness of the water used for dilution.

Emulsifiable concentrates gave some real problems, many of them closely allied to those of suspensibility. Early observations indicated that the specific gravity of the mixture (insecticide, solvent, and emulsifier) was of importance. If the weight was less than that of water, the droplets came to the surface; if heavier, they settled to the bottom. If the mixture came to the surface, wind and sun evaporated the solvent, changing the basic formula and decreasing the liklihood that reemulsification would occur. There was also obviously a greater concentration of insecticide in the floating layer and a decrease in the water below it. In a dipping vat (bath), a submersed animal, on returning to the surface, could be coated with a heavy dosage of insecticide. Uneven control of parasites was inevitable. If in a spray device with delivery from the bottom, the first treatments were unusually light, the last unusually heavy, the latter leading the livestock poisoning.

On the other hand, when specific gravity was high, the droplets settled out in a layer along the bottom. Settling in dipping vats, along with assorted dirt, hair, and debris, the

particles could coalesce. In some cases, the coalescense was followed by complete separation of the insecticide from the solvent or emulsifier. When this occurred, there was no reemulsification, resulting in total failure of parasite control, but usually in complete safety for the dipped animals. The intermediate stages of this deterioration were extremely dangerous for livestock. We learned that adhesion of emulsion droplets to animal hair was related directly to the size of the droplets (112). Greatest uniformity in treatment was obtainable with droplets less than 8 microns in diameter (same diameter as our red blood cells). Indeed, best work could be done with near-colloidal emulsions. A change in particle size from 4 to 8 microns increases deposition twofold. Particles at 60 to 150 microns increase deposition as much as 30 times.

Thus it was that a dipping vat could be charged with a dilution containing a certain percentage of insecticide. If the particles were of 1 to 5 microns diameter, 1 unit of insecticide would be deposited on the hair, while if the particle size increased to 8 microns, 2 units would be found on the hair. At 60 to 120 microns, 30 units would be deposited. Using a compound wherein 1 unit deposition was safe, but 2 dangerous, one can readily see that the increase in size was a serious danger to livestock.

The particle size effect was greatest in dipping operations, because the emulsions were held for months at a time and the dipped animals were exposed to hundreds or thousands of gallons of emulsion. Spraying usually involved the application of 1 or 2 gallons per head, and each few hours of operation meant the preparation of new material. Deterioration with time was unimportant unless the operator was an unduly thrifty man who tried to save material for future treatments.

Efforts have been made by manufacturers to produce the finer quality emulsifiables. Most materials marketed for livestock use at the present time have been carefully formulated. Most of these are recommended only for spray use because few manufacturers have been able to develop formulations that can stand without deterioration in dipping vats.

The early recognition of these effects prevented many unfortunate accidents and insured uniform parasite control.

Misunderstanding apparently still exists about the selection of proper formulations for animal treatment. Our manufacturers produce certain materials for animal use and others for plant use. These are by no means interchangeable from plants to animals. Occasionally a material is made for both purposes, but these are of the minority. It is important that users of animal insecticides read the labels on these materials and assure themselves of proper usage.

Effect of Emaciation and Lactation

We found that emaciation and lactation, singly or in combination, predisposed sheep, (89), and probably cattle, to poisoning by certain of the chlorinated hydrocarbons, notably the gamma isomer of BHC, but including toxaphene and chlordane.

Effect of Stress

While emaciation and lactation produce an increased susceptibility to poisoning, the opposite effect was noted with Bayer L 13/59 (Dipterex) during our studies of that compound as a systemic insecticide. One demonstration was inadvertant. We selected an intractable sheep to demonstrate kill of screwworms. Following treatment, the sheep (restrained by a neck rope) struggled extensively to escape. Although the dosage of Dipterex was twice that ordinarily required for screwworm kill, none of the worms were destroyed.

This peculiarity was studied further under controlled conditions, using a radioactive form of Dipterex in rabbits.[2] Again, when the rabbits were under stress, struggling against restraint, neither stableflies nor screwworms were destroyed. When doses ordinarily toxic for the rabbit were given, no symptoms appeared.

G. W. Eddy of the ARS Corvallis, Oreg., Laboratory appeared to have encountered the same phenomenon in cattle treated for cattle grub control.[3] The cattle were chased deliberately before treatment by the cowboys to wear them down to a more tractable disposition. The cattle grub control was most unsatisfactory in this group.

These casual observations of specific results of stress serve to warn us of possible additional effects. Toxicity studies are generally conducted with healthy animals, usually free of stress, and usually with the single operation of administering the compound being carried out.

When the same material is given to sick animals, to those castrated, dehorned, vaccinated, and drenched in the same day, the results may be far different. It is not possible to predetermine all the possible combinations animal owners may devise; therefore, we suggest that pesticide usage should follow or preceded such drastic stress by several days.

Problems with Systemic Insecticides

A new area of toxicology was opened by the development of systemically active compounds for plant and animal usage. The same problem existed for these materials as for the earlier ones, but new problems were added. Systemics were found to be changed within plants and animals to other related compounds, which might be more toxic to insects and livestock than the parent compound (81,102,103). Indeed, in most cases, some metabolic breakdown appeared necessary for the development of the systemic action. Careful study of this metabolism has given us leads for new insecticides, more effective timing of treatment, and recognition of the limitations of the method.

The study of pesticide toxicology has thus become a highly complicated science, requiring the services of several disciplines. It has not been sufficient to simply determine a toxic dose for these materials. We have discussed the problems with those compounds on several occasions (91,93,100).

The transformation of a chemical in plants or animals to substances other than the originally applied material (that is, schradan, thimet, and others) (13,23,76,117) complicates the evaluation of its potential hazard, particularly from the standpoint of residue in tissues, since we must not only evaluate the hazard of the compound applied, but of the new compounds derived from it. Additional complications occur because of the occasional unavailability of these derived compounds in sufficient quantity for animal experimentation.

The problem is not entirely limited to those materials considered to be systemically active. Aldrin and heptachlor are examples. These compounds, when absorbed by mammals, are biologically converted to their epoxides, dieldrin and heptachlor epoxide, and analyses must be performed for these compounds rather than aldrin or heptachlor.

HERBICIDES

The purpose of herbicides--killing of plants--limits the palatability of the treated vegetation in many cases, and reduces the period of attractiveness of the feeds to a very few weeks. The danger is primarily that of consuming the freshly treated plants.

[2] Unpublished results.
[3] Private communication.

With a few exceptions, the modern herbicides are of a relatively low order of toxicity, thus limiting the hazard of their use.

A number of researchers have reported upon the toxicity of 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichloro-phenoxyacetic acid), and their esters (38,53, 63,77,107,108). The studies of Mitchell, et al, (1946) and of Grigsby and Farwell (1950), in which they grazed livestock on heavily treated pastures, indicate that the practical hazard with these materials is very low. Rowe and Hymas (1954) have studied 2,4,5-T by administering it to steers.

At Kerrville, we have studied several herbicides in sheep and cattle. (Table 4).

It is apparent that pentachlorophenol is one of the more toxic of these materials, producing poisoning in cattle and sheep in single doses of 25 mg./kg. Of this group, dinitro-o-sec-butyl phenol is the next most toxic, proving lethal for sheep after 4 daily doses of 25 mg./kg. Oddly, the alkanolamine salts of dinitro-o-sec-butyl phenol are much less toxic, one sheep having tolerated 35 daily doses of 25 mg./kg. each without showing symptoms.

In general, the phenoxy-acid group of herbicides was of very low toxicity for sheep. The most toxic of this group was 2-(2,4,5-trichlorophenoxy) propionic acid in the form of propylene glycol butyl ether esters, 11 daily doses of 100 mg./kg. each producing death. This would still class it as a relatively nontoxic compound.

The following materials were nontoxic when administered daily in 100 mg./kg. doses for 35 days: The alkanolamine salts of 2,4-dichlorophenoxyacetic acid and of 2-methyl-4-chlorophenoxyacetic acid, the propylene glycol butyl ether esters of 2,4-dichlorophenoxyacetic acid and of 2,4,5-trichlorophenoxyacetic acid, the triethylamine salts of 2,4,5-trichlorophenoxyacetic acid and the sodium salt of 2,2-dichloropropionic acid.

The absence of poisoning from these materials administered in such high dosages for 5 weeks indicates that there is little hazard to livestock to be expected from normal usage.

Dehydroabietylamine acetate (Delrad), an algaecide based upon technical abietalamines, is used at very low rates in reservoirs and irrigation canals. Cattle and sheep were unharmed by consuming water treated with 100 p.p.m. Delrad, which is several times the concentration usually employed. This compound is extremely distasteful to cattle and sheep and probably would never be consumed by them voluntarily even if the chemical were left exposed. Cattle were unharmed by doses of 200 mg./kg., sheep by doses of 250 mg./kg. Higher doses produced poisoning.

Because of the low toxicity and reduced hazard of herbicides, together with comparatively restricted usage, the number of publications is small. Much of the evaluation of toxicity of these materials has been done with laboratory animals rather than with livestock.

Reduction of Hazard by Control of Toxicity

While very little can be done to change the specific toxicity of a pesticide, several things can be done to control it.

There are certain compounds which may be administered simultaneously with the pesticide which antagonize its action directly or slow its action sufficiently to enable the organism to deal with it.

TABLE 4.--Summary of studies of the oral toxicity of herbicides to sheep and cattle at Kerrville, Tex.

Chemical	Animal	Dose	Frequency	Results
		mg./kg.		
Pentachlorophenol	Sheep	25	1 dose	mild toxicity
	Cattle	25	1 dose	mild toxicity
(Delrad) Dehydroabietylamine acetate	Sheep	250	1 dose	tolerated, higher doses toxic
	Cattle	200	1 dose	tolerated, higher doses toxic
Dinitro-o-sec-butyl phenol	Sheep	25	daily	death after 4 doses
Alkanolamine salts of Dinitro-o-sec-butyl phenol	Sheep	25	daily	tolerated 35 doses*
2-(2,4,5-Trichlorophenoxy) propionic acid, propylene glycol butyl ether esters	Sheep	100	daily	lethal after 11 doses
2-4-Dichlorophenoxyacetic acid, alkanolamine salts	Sheep	100	daily	tolerated 35 doses*
2,4,5-Trichlorophenoxyacetic acid, alkanolamine salts	Sheep	100	daily	tolerated 35 doses*
2,4-Dichlorophenoxyacetic acid, propylene glycol butyl ether esters	Sheep	100	daily	tolerated 35 doses*
2,4,5-Trichlorophenoxyacetic acid, propylene glycol butyl ether esters	Sheep	100	daily	tolerated 35 doses*
2,4,5-Trichlorophenoxyacetic acid, triethylamine salts	Sheep	100	daily	tolerated 35 doses*
2,2-Dichloropropionic acid, sodium salt	Sheep	100	daily	tolerated 35 doses*

*At time of preparation of this table, these tests were in progress.

Pesticides prepared in granular form for many uses accomplish their objective more effectively; yet, because of the weight of the granules, the amount remaining on foliage or drifting away from the area is greatly reduced.

Biological Cycles

To a certain extent we have biological cycles involved in the use of pesticides. For example, we may treat a body of water for insect control and accomplish the objective, but organisms not killed by the treatment may absorb and store the chemical. They, in turn, according to their biological position, may be consumed as food by other species, and these in turn by others, until there is ultimately a removal from that water of a species serving as food for mammals or birds, or man. The cycle is then continued outside the original environment.

The accumulation of pesticide may be attenuated or concentrated within this cycle, depending upon the part the animal or plant may provide of the total diet of the consum-

ing animal. The principle has been established by several workers (22,52,55) in addition to the studies involving contamination of the ordinary daily diet.

Symptoms of Poisoning

Several workers have described the symptoms of insecticide poisoning (12,15,17,24, 44,48,49,62,66,68,69,74,80,83,84,86,89,90,95,96,97,98,99,100,113,121).

Chlorinated Hydrocarbons.--These insecticides stimulate the central nervous system, producing neuro-muscular symptoms of extreme variety. Initial symptoms are an increased awareness of the animal to its surroundings. Muscular twitching and spasms are then observed, usually beginning at the head and progressing posteriorly.

In poisoning by DDT, TDE, and methoxychlor, the muscular spasms become simultaneously generalized, causing the animal to appear to be shivering with cold. With other members of this group, the spasms are usually followed by convulsive seizures of a clonic-tonic type, with or without the intermediate trembling. The convulsions may last for a few seconds or several hours and may be continuous or repeated at intervals.

Abnormal posturing is often seen. Occasionally an animal becomes belligerent. There is generally a flow of thick saliva and frequently dribbling of urine. Outcrying is common.

During convulsive seizures, the body temperature may raise to 114° to 118°F.

Certain poisoned animals fail to show the more active symptoms and are depressed and appear to be almost completely lost from their environment. They fail to eat or drink. These animals usually live for a few more days than those violently affected, but the death rate is about the same.

In certain cases, the symptoms alternate between violence and depression.

The severity of symptoms in a given animal is difficult to translate into a prognosis. Some animals suffer a single convulsion and die, while others survive innumerable seizures.

While the symptoms observed are rather definite, they are not definitive enough to be diagnostic. Many afflictions of the central nervous system can duplicate the symptoms of poisoning by these insecticides.

Organic Phosphorus Compounds.--This group of compounds is generally considered to produce poisoning by inhibition or interference with the activity of the enzyme cholinesterase, primarily in the nervous system. There is good evidence that poisoning may exist due to fractions of the molecules not having an affinity for the enzyme.

When intoxication is primarily due to enzyme interference, the clinical symptoms are primarily cholinergic, including mild to profuse salivation, dyspnea, signs of abdominal pain, ataxia, diarrhea, and, occasionally, convulsions.

Onset of symptoms may be within 5 to 6 minutes of treatment, or may be delayed for 2 to 3 days, according to the compound and dosage involved. The course of intoxication is governed by the compound and dosage. Materials such as malathion, diazinon, Bayer L 13/59, parathion, and TEPP produce poisoning of short duration, resulting in death or recovery within 24 hours of exposure. Ronnel and Bayer 21/199 produce their effects over a much longer period of time and may show effects of the intoxication for 30 days or more.

These materials decrease the activity of cholinesterase in blood as well as in nerve tissue, and the blood is usually used as an indicator of the amount of the enzyme in the

nervous tissue. Unfortunately, this indicator often fails because animals can die with high levels of the enzyme in the blood while others live quite normally with none in the blood.

Carbamates.--Insecticides of this group are cholinesterase inhibitors but are generally not so active in this regard. Symptoms of poisoning generally parallel those produced by the organic phosphorus compounds, with a few additions. Usually the ataxia is more pronounced. The poisoned animal generally attempts to move rapidly, but succeeds only in rolling end-over-end in a tumbling manner, recovers rapidly, and repeats the process. There is usually a great deal of trembling and shaking. The poisoning by carbamates follows the general course described for the organic phosphorus compounds.

Lesions of Poisoning

Chlorinated Hydrocarbons.--Acute poisoning by these compounds yields no pathognomonic lesions. The lesions found are those expected in violent deaths from other causes--cloudy swelling, blanched viscera, and hemorrhages on various organs. In addition, there may occasionally be congestion of the lungs. The heart is generally found to be in systole.

Subacute and chronic exposure lead to degenerative changes in the liver and kidneys.

Organic Phosphorus Compounds and Carbamates.--This group usually leaves the viscera unmarked. Certainly there are no diagnostic lesions. Occasionally the lungs are edematous or congested, and occasionally there may be congestion with hemorrhages in various viscera.

Herbicides.--The dinitro herbicides create severe damage in the kidneys and liver. The lungs are gray in color. Often there is a yellow discoloration of all tissues and frequently the odor of the compound is easily detected.

The 2,-(2,4,5-trichlorophenoxy) propionic acid salts produce degenerative changes in the liver and kidneys and rather spectacular hemorrhages on the surface of the heart.

The other phenoxy-acid compounds are reported to produce kidney and liver damage.

Diagnosis of Poisoning

Proper diagnosis of suspected poisoning of livestock has been a major concern of ARS. To aid in this problem, we have developed films describing and pointing out methods of arriving at correct diagnosis.

Differential diagnosis was not, and is not, easy in the case of chlorinated hydrocarbon pesticides. The symptoms mimic those of many diseases in which the central or peripheral nervous structures are afflicted. In many cases, a diagnosis can be reached only by eliminating all other possible conditions. Proper diagnosis are reached only after careful evaluation of the history of exposure, symptoms, and the lesions (the latter being nonpathognomonic in acute chlorinated hydrocarbon poisoning, but may suggest other disease processes).

Differential diagnosis of poisoning by other pesticides is not appreciably easier. All must be approached with caution and carefully studied.

Use of Cholinesterase Determinations in Diagnosis.--The organic phosphorus insecticides and the newer carbamates are recognized as inhibitors of cholinesterase, either as the original compound or as metabolites of the original.

Originally, we (92), and others, felt that blood cholinesterase activity would be an excellent indicator of exposure and of poisoning. For single exposures we feel the method

applicable to certain compounds, such as parathion, diazion, malathion, and Bayer L 13/59. With these materials a rapid drop of blood cholinesterase to 10 percent or so of normal is usually accompanied by symptoms of poisoning.

For repeated exposures, the method loses significance. With parathion we have reduced blood cholinesterase activity to zero with 2 doses of 20 mg./kg. in cattle, but poisoning as manifest by symptoms, did not become evident until after 78 additional daily doses of 20 mg./kg. each.

Zero activity levels repeatedly with Co-Ral (Bayer 21/199) and with other insecticides, which persisted for several weeks without the appearance of symptoms, have been observed.

Several of the new experimental insecticides produce cholinergic symptoms and even death while cholinesterase activity in blood is near 50 percent of normal. This is not helpful in reaching a diagnosis of poisoning. Hazelton pointed out similar observations (59).

There is safety in using cholinesterase activity to indicate exposure to organic phosphorus compounds or carbamates, but we believe this is the only firm conclusion that can be drawn.

Residues in Diagnosis.--The use of chemical or biological determinations in the diagnosis of poisoning by insecticides is vastly complicated by their behaviour.

I doubt that sufficient emphasis can be given to this problem. It is not readily understood because the necessary explanations are apparently contradictory of our usual concept of the significance of the presence of a foreign chemical in animal or human tissues. This lack of understanding has caused us particular difficulty when toxicologists, after finding high residues, flatly state that these residues indicate death due to the material found.

Let me carry you through some examples. In feeding tests at Kerrville with heptachlor, using a diet containing 60 p.p.m. of heptachlor, 52 p.p.m. of heptachlor epoxide could be recovered from the fat of cattle at the end of a 16-week feeding period. The cattle at that time were in excellent health and condition. One of the cattle, when slaughtered immediately after the feeding period, contained, as stated, 52 p.p.m. in the fat, 1.2 p.p.m. in the thymus, and 1.5 in the liver. Other tissues, including brain, kidney, muscle, adrenal, heart, lung, and spleen contained none.

By contrast, a calf poisoned and killed by a single large dose of heptachlor, revealed a residue of only 2.2 p.p.m. in its fat.

In our feeding trials with lindane, using a level of 100 p.p.m. in the feed for 10 weeks, a residue of 100 p.p.m. existed in the fat at the end of the feeding period. This was reduced to 50 p.p.m. in 4 weeks on control feed.

By contrast, three cattle of similar breed, age, and condition, deliberately poisoned by high concentrations of lindane in dips, revealed only 23 p.p.m. 1 week later in their fat.

Let us assume that either of these animals with high residues had been found dead on pasture treated with the particular insecticide, no history other than this available and with the animals having been seen in good health a few days earlier. At necropsy, no lesions are observed other than a few petechiae on the heart and cloudy swelling of various organs. Because of the prior use of insecticides, tissues are taken for analysis, with the results being at high levels. Unfortunately, many veterinarians would accept this as definite evidence of death caused by the insecticide and so render their diagnosis. Some have, only to have others prove the presence of rabies, Aujesky's disease, or other diseases.

On the other hand, had the residue of 2.2 p.p.m. been reported, it would have lacked impressiveness and a diagnosis of some other condition may have been made, erroneously clearing the insecticide.

We have, in the past, suggested that chemical analyses be performed on hair or ingesta and that large quantities present would indicate poisoning. That has been altered by several complications. We now know, from a study with lindane, that low residues on hair may exist in poisoning. We also are at a loss to specify the quantity of compound in ingesta which will or would result in poisoning. Bioassays of hair or gastrointestinal content would yield grossly misleading results.

Organic phosphorus compounds may or may not leave detectable residues, according to the material. The use of chemical analyses with these materials is as confusing as it is with chlorinated hydrocarbons.

We emphasize that chemical analyses usually serve to confuse rather than inform when we attempt to use them as an aid in diagnosis.

Problems in Sampling

There would be no need to emphasize this point, but samples continue to be shipped in such a manner as to either confuse the chemist or make analysis impossible.

The need for fresh tissue in quantities as near 500 gms., if the organ permits, free of preservatives and contaminants and properly refrigerated cannot be over emphasized.

If fresh tissue is not always available, one should not expect the chemist in turn to be as good in his analysis.

Samples of tissues weighing only a few grams are rarely enough for a single analysis, much less for duplicates or for analyses for more than one substance.

Preservatives should never be used, because they may easily mask the reactions desired.

Freezing is preferred in all cases. Shipment should be in liberal quantities of dry ice and by air or messenger. Most chemists prefer polyethylene bags as sample containers. Cartons, boxes, and bottles may contain waxes or finishes which confuse the analysis.

Otherwise satisfactory samples are often ruined by the presence of antiseptics or soaps from gloves and hands or insecticide from hair or dirt blown or carried into the sample.

Because the final reaction in the analysis may be for an entirely different molecule--for example benzene in the lindane analysis or sodium chloride for some chlorinated hydrocarbons, the presence of all extraneous chemicals must be avoided.

The chemist should be informed of the insecticide suspected, but here another difficulty is encountered. Animals fed treated forage or feed will show some residue in their fat in almost all cases. Therefore, finding a residue is not conclusive evidence. The person submitting the sample should keep his mind open to all other chemicals, including sprays and dips, to which the animal may have been exposed.

Unless the tissues are analyzed for all possible substances, the finding of one simply leads to erroneous conclusions.

Above all, the presence of a residue of insecticide must never be taken to indicate anything more than exposure to that material. Presence of a residue must never be taken as a diagnosis of poisoning.

Treatment

To the farmer with poisoned livestock, nothing is more important than saving those afflicted and those exposed. Unfortunately, few of our pesticides have specific antidotes. In general, treatment must consist of elimination of the poison by washing or purging and control of the signs and symptoms that may occur.

Metallic poisons can be treated to some degree--that is, arsenical poisoning by BAL, and lead by EDTA--but the success of treatment lies in early recognition. The metallic poisons induce changes of essential organs that are largely irreversible; therefore, if the condition has existed for an appreciable time, successful treatment leaves an inefficient animal that is more of a liability than an asset.

Oddly enough, most organic pesticidal compounds produce changes which are reversible until the animal reaches the extreme stages of poisoning. This is true of acute insecticide poisoning in particular. Removal of poison and symptomatic treatment often restores livestock poisoned with chlorinated hydrocarbons to full health within a matter of a few hours. In the case of animals poisoned by organophosphorus compounds, essential normalcy may be restored in a matter of less than 10 minutes following atropine therapy.

Recoveries are apparently full and complete, without evidence of impairment of any essential function.

This lack of permanent damage is certainly to be considered in choosing between pesticides for safety. Compounds that is arsenic showing such reversibility are without question more desirable than those producing permanent damage even though the animal survives.

CONCLUSION

In our discussion we have covered the relationship of toxicity to hazard and have shown that a few pesticides are so highly toxic as to be hazardous in use unless handled by skilled people, but that the majority are not hazardous to livestock under the conditions of ordinary usage or even if misused to a moderate extent.

We have shown that residues of pesticides on forage are practically never a hazard to livestock, but that some of the pesticides are stored in the tissues of livestock and excreted in their milk, creating a potential hazard to people. This hazard can be avoided by carefully following label instructions as to dosage and proper intervals which must occur between treatment and harvest or slaughter.

Various factors affecting the toxicity and hazard of pesticides to livestock, such as age, species, formulation, emaciation, lactation, and stress have been discussed.

The symptoms, lesions, diagnosis, and treatment of poisoning by the various groups of pesticides have been discussed, showing the difficulties faced by a veterinarian in reaching a sound diagnosis.

We have pointed out that all the toxicological information developed for a given compound can be utilized only if the manufacturer, formulator, and consumer contribute equally to safe, intelligent usage. The manufacturers and formulators must prepare the compounds in stable, readily usable form, and devise labels for the final product, which will clearly define the intended uses and limitations of the product.

No matter how carefully the product may have been prepared and labeled, and no matter how complete the scientific background may be, the consumer or user may nullify these totally and completely and produce a catastrophic result to his animals, plants, or himself. It seems silly to say that the most important consideration in the safe use of pesticides is the ability of the user to read and follow the label instructions;

yet we are constantly reminded that this is the weak link in our safety chain, and that by far the majority of cases of poisoning of all kinds of people, animals, and plants are due simply to carelessness or unwillingness to follow the directions.

We believe that the use of pesticides in agriculture is essential if we are to maintain maximum productivity in the face of constantly shrinking farm and ranch land and an exploding population. We believe that these materials can be, and are being, used with complete safety to man and animals when label instructions are followed.

REFERENCES

1. Allen N. N., Lardy, H. W., and Wilson, H. F. (1946) The effect of ingestion of DDT upon dairy cows. J. Dairy Science 29(8): 530-531.

2. Anderson, R. W., Blakely, R. M., and MacGregor, H. I. (1951) The effect of aldrin on growing turkeys. Poultry Science 30: 905.

3. App, B. A., Carter, R. H., and Ely, R. E. (1956) Residues in forage, in the soil, and in milk following pasture treatment with granulated dieldrin. Jour. Econ. Ent. 49(1): 136-137.

4. Arant, F. S. (1952) Toxicity of aldrin to chickens. Jour. Econ. Ent. 45(1): 121.

5. Baker, N. F., Allen, P. H., and Douglas, J. R. (1959) Trial with a new organic phosphate as an anthelmintic in cattle. Am. J. Vet. Res. 20(75): 278-280.

6. Bann, James M., DeCino, Thomas J., Earle, Norman W., and Sun, Yun Pei. (1956) The fate of aldrin and dieldrin in the animal body. Jour. Agri. & Food Chem. 4(11): 937-941.

7. Bateman, G. Q., Biddulph, Clyde, Harris, J. R., Greenwood, D. A., and Harris, L. E. (1953) Transmission studies of milk of dairy cows fed toxaphene-treated hay. Jour. Agri. & Food Chem. 1(4): 321-324.

8. Batte, E. G., and Turk, R. D. (1948) Toxicity of some synthetic insecticides. Jour. Econ. Ent. 41(1): 102-103.

9. Biddulph, C., Bateman, G. Q., Bryson, M. J., Harris, J. R., Greenwood, D. A., Binns, W., Miner, M. L., Harris, L. E., and Madsen, L. L. (1950) DDT in milk and tissues of dairy cows fed DDT dusted alfalfa hay. Adv. in Chem., Series 1: 237-243.

10. Biddulph, C., Bateman, G. Q., Harris, J. R., Mangelson, F. L., Lieberman, F. V., Binns, W., and Greenwood, D. A. (1952) Effect of feeding methoxychlor-treated alfalfa hay to dairy cows. J. Dairy Science 35(5): 445-448.

11. Bohman, V. R., Chi, I. A., Harris, L. E., Binns, W., and Madsen, L. L. (1952) The effect of DDT upon the digestion and utilization of certain nutrients by dairy calves. J. Dairy Science 35(1): 6-12.

12. Borgman, R., Symptoms of poisoning by insecticides. (1951) Vet. Med. 46(6): 237.

13. Bowman, J. S., and Casida, J. E. (1957) Metabolism of the synthetic insecticide O,O,diethyl S-ethylthiomethyl phosphorodithioate (Thimet) in plants. Jour. Agr. & Food Chem. 5(3): 192-197.

14. Bryson, M. J., Draper, C. I., Harris, J. R., Biddulph, C., Greenwood, D. A., Harris, L. E., Binns, W., Miner, M. L., and Madsen, L. L. (1950) DDT in eggs and tissues of chickens fed varying levels of DDT. Adv. in Chem., Series 1: 232-236.

15. Buck, William B., Radeleff, R. D., Jackson, J. B., Claborn, H. V., and Ivey, M. C. (1959) Oral toxicity studies with heptachlor and heptachlor epoxide in young calves. Jour. Econ. Ent. 52(6): 1127-1129.

16. Bushland, R. C., Claborn, H. V., Beckman, H. F., Radeleff, R. D., and Wells, R. W. (1950) Contamination of meat and milk by chlorinated hydrocarbon insecticides used for livestock pest control. Jour. Econ. Ent. 43(5): 649-652.

17. Bushland, R. C., Wells, R. W., and Radeleff, R. D. (1948) Effect on livestock of sprays and dips containing new chlorinated insecticides. Jour. Econ. Ent. 41(4): 642-645.

18. Carter, R. H., Hubanks, P. E., Poos, F. W., Moore, L. A., and Ely, R. E. (1953) The toxaphene and chlordane content of milk from cows receiving these materials in their feed. Jour. Dairy Science 36: 1172-1177.

19. Carter, R. H., Wells, R. W., Radeleff, R. D., Smith, C. L., Hubanks, P. E., and Mann, H. D. (1949) The chlorinated hydrocarbon content of milk from cattle sprayed for control of hornflies. Jour. Econ. Ent. 42(1): 116-118.

20. Carter, R. H., and Mann, H. D. (1949) The DDT content of milk from a cow sprayed with DDT. Jour. Econ. Ent. 42(4): 708.

21. Carter, R. H., Hubanks, P. E., and Mann, H. D. (1948) Effect of cooking on the DDT content of beef. Science 107(2779): 347.

22. Carter, R. H., Hubanks, P. E., Mann, H. D., Zeller, J. H., and Hankins, O. G. (1948) The storage of DDT in the tissues of pigs fed beef containing this compound. J. Anim. Science 7(4): 509-510.

23. Casida, John E., and Stahmann, Mark A. (1953) Metabolism and mode of action of Schradan. J. Agr. & Food Chem. (14): 883-888.

24. Choudhury, B., and Robinson, V. B. (1950) Clinical and pathological effects produced in goats by the ingestion of toxic amounts of chlordane and toxaphene. Am. J. Vet. Res. 11(38): 50-57.

25. Claborn, H. V., Bowers, J. W., Wells, R. W., Radeleff, R. D., and Nickerson, W. J. (1953) Meat contamination from pesticides. Agr. Chem. 8(8): 37-39, 119, 121.

26. Claborn, H. V., and Wells, R. W. (1952) Methoxychlor, DDT, CS-708 and Dieldrin--their rates of excretion in milk. Agr. Chem. 7(10): 28-29, 115.

27. Claborn, H. V., Radeleff, R. D., Beckman, H. F., and Woodard, G. T. (1956) Malathion in milk and fat from sprayed cattle. Agr. & Food Chem. 4(11): 941-942.

28. Claborn, H. V. (1956) Insecticide residues in meat and milk. U. S. Dept. Agr. ARS 33-25.

29. Dahm, Paul A., and Jacobson, Normal L. (1956) Effects of feeding Systox-treated alfalfa hay to dairy cows. J. Agr. & Food Chem. 4(2): 150-155.

30. Davich, T. B., Tombes, A. S., and Carter, R. H. (1957) Insecticide control of green june beetle larvae attacking ladino clover pastures: Residues on foliage and accumulation in swine tissue. J. Econ. Ent. 50(1): 97-100.

31. Davidow, B., and Frawley, J. P. (1951) Tissue distribution, accumulation and elimination of the isomers of benzene hexachloride. Proc. Soc. Exp. Biol. Med. 76: 780-783.

32. Davidow, B., Hagan, E. C., and Radomski, J. L. (1951) A metabolite of chlordane in tissues of animals. Fed. Proc. (Amer. Soc. Exp. Biol.) 10: 291.

33. Davidow, B., and Radomski, J. L. (1952) Metabolite of heptachlor, its analysis, storage, and toxicity. Fed. Proc. (Amer. Soc. Exp. Biol.) 11: 336.

34. Davidow, B., and Radomski, J. L. (1953) Isolation of an epoxide metabolite from fat tissues of dogs fed heptachlor. J. Pharmacol. Exp. Therap. 107: 259-265.

35. Davidow, B., Radomski, J. L., and Ely, R. (1953) Excretion of heptachlor epoxide in milk of a dairy cow fed haptachlor. Science 118: 383-384.

36. Douglas, J. R., Baker, N. F., and Allen, P. H. (1959) Trial with a new organic phosphate as an anthelmintic in sheep. Am. J. Vet. Res. 20(76): 442-444.

37. Draper, C. I., Harris, J. R., Greenwood, D. A., Biddulph, C., Harris, L. E., Mangelson, F., Binns, W., and Miner, M. L. (1952) The transfer of DDT from the feed to eggs and body tissues of White Leghorn hens. Poultry Science 31(3): 388-393.

38. Drill, V. A., and Hiratzka, T. (1953) Toxicity of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. A report of their acute and chronic toxicity in dogs. Arch. Ind. Hyg. and Occup. Med. 7: 61-67.

39. Ely, R. E., Moore, L. A., Carter, R. H., and Poos, F. W. (1949) The DDT, Toxaphene, and Chlordane content of milk as affected by feeding alfalfa sprayed with these insecticides. U. S. Dept. Agr. Res. Administ. BDIM-Inf. 85, 4 pp.

40. Ely, R. E., Moore, L. A., Hubanks. P. E., Carter, R. H., and Poos, F. W. (1954) Studies of feeding aldrin to dairy cows. J. Dairy Science 37: 294-298.

41. Ely, R. E., Moore, L. A., Hubanks, P. E., Carter, R. H., and Poos, F. W. (1955) Excretion of heptachlor epoxide in the milk of dairy cows fed heptachlor-sprayed forage and technical heptachlor. J. Dairy Science 38: 669-672.

42. Ely, Ray E., Moore, L. A., Carter, R. H., Mann, H. D., and Poos, F. W. (1952) The effect of dosage level and various methods of administration on the concentration of DDT in milk. Jour. of Dairy Science 35: 266-271.

43. Ely, Ray E., Moore, L. A., Mann, H. D., and Carter, R. H. (1952) The effect of various dosage levels of crystalline lindane on the concentration of lindane in cow's milk. Jour. of Dairy Science 35: 733-737.

44. Ely, Ray E., Underwood, P. C., Moore, L. A., Mann, H. D., and Carter, R. H. (1953) Observations on lindane poisoning in dairy animals. J.A.V.M.A. 123(920): 448-449.

45. Fahey, J. E., Brindley, T. A., and Spear, M. L. (1955) DDT residues in fat from steers pastured on corn stover in DDT-treated fields. J. Econ. Ent. 48(5): 606-607.

46. Furman, D. P., and Bankowski, R. A. (1949) Absorption of BHC in poultry. J. Econ. Ent. 42(6): 980-982.

47. Furman, D. P., and Hoskins, W. M. (1948) BHC in cream from cow's milk. J. Econ. Ent. 41(1): 106-107.

48. Gaafar, S. M., Turk, R. D. (1957) Toxicity of malathion in chickens. Vet. Res. 18(66): 180-182.

49. Galvin, T. J., Bell, R. R., and Turk, R. D. (1959) The efficacy and toxicity of certain organic phosphates and a carbamide as anthelmintics in ruminants. Amer. J. Vet. Res. 20(78): 784-787.

50. Gjullin, C. M., Scudder, H. I., and Erwin, W. R. (1955) Determination of malathion and its influence on flavor of milk from cows fed malathion-sprayed alfalfa. J. Agri. & Food Chem. 3(6): 508-509.

51. Gordon, H. McL. (1958) Studies on anthelmintics for sheep. Some organic phosphorus compounds. Aust. Vet. Jour. 34: 104.

52. Greenwood, D. A., Harris, L. E., Biddulph, C., Bateman, G. Q., Binns, W., Miner, M. L., Harris, J. R., Mangelson, F., and Madsen, L. L. (1953) Feeding rats tissues from lambs and butterfat from cows that consumed DDT-dusted alfalfa hay. Proc. Soc. for Exptl. Biol. & Med. 83: 458-460.

53. Grigsby, B. H., and Farwell, E. D. (1950) Some effects of herbicides on pasture and on grazing livestock. Michigan State Univ. Agr. Expt. Sta. Quart. Bull. 32: 378-385.

54. Gyrisco, George G., Norton, L. B., Trimberger, G. W., Holland, R. F., McEnerny, P. J., and Muka, A. A. (1959) Effects of feeding low levels of insecticide residues on hay to dairy cattle on flavor and residues in milk. J. Agr. & Food Chem. 7(10): 707-711.

55. Harris, Lorin E., Harris, Joseph R., Mangelson, Farrin L., Greenwood, Delbert A., Biddulph, Clyde, Binns, Wayne, and Miner, Merthyr L. (1953) Effect of feeding DDT-treated alfalfa hay to swine and of feeding the swine tissues to rats. Jour. of Nutrition 51(4): 491-505.

56. Harris, Joseph R., Biddulph, Clyde, Greenwood, D. A., Bryson, Melvin J., Binns, Wayne, Miner, M. L., and Madsen, Louis L. (1949) Concentration of DDT in the blood and tissues of sheep fed varying levels of DDT. Arch. of Biochem. 21(2): 370-376.

57. Harris, L. E., Myint, T., Biddulph, C., Greenwood, D. A., Binns, W., Miner, M. L., and Madsen, L. L. (1951) Effect of feeding DDT-dusted alfalfa hay to fattening lambs. Jour. Animal Science 10(3): 581-591.

58. Harris, Joseph R., Stoddard, George E., Bateman, George Q., Shupe, J. LeGrande, Greenwood, Delbert A., Harris, Lorrin E., Bahler, Homas L., and Lieberman, Frank V. (1956) Effects of feeding dieldrin- and heptachlor-treated alfalfa hay to dairy cows. J. Agr. & Food Chem. 4(8): 694-696.

59. Hazelton, L. W. (1956) Review of current knowledge of toxicity of cholinesterase inhibitor insecticides. J. Agr. & Food Chem. 4(5): 312-319.

60. Helrich, Kenneth, Hansens, Elton J., and Granett, Philip. (1958) Residues in milk from dairy cattle treated with methoxychlor for fly control. J. Agr. & Food Chem. 6(4): 281-283.

61. Herlich, H., and Johnson, J. M. (1957) Critical tests on the efficiency of Dow ET-57 as an anthelmintic in cattle. J. Parasitol. 43: 19.

62. Herlich, H., and Porter, D. A. (1958) An anthelmintic for cattle and sheep. Critical tests of efficacy of Bayer 21/199. Vet. Med. 53(7): 343-348, 360.

63. Hill, E. C., and Carlisle, H. (1947) Toxicity of 2,4-dichlorophenoxyacetic acid for experimental animals. J. Ind. Hyg. & Tox. 29: 85-95.

64. Howell, D. E., Cave, H. W., Heller, V. G., and Gross, W. G. (1947) The amount of DDT found in the milk of cows following spraying. J. Dairy Science 30: 717-721.

65. Jackson, J. B., Ivey, M. C., Roberts, R. H., and Radeleff, R. D. (1959) Residue studies in sheep and goats dipped in 0.025% lindane. J. Econ. Ent. 52(5): 1030-1031.

66. Jolly, D. W. (1954) Studies in the acute toxicity of dieldrin to sheep. Vet. Rec. London 66: 444-447.

67. Kiigemagi, U., Sprowls, R. G., and Terriere, L. C. (1958) Endrin content of milk and body tissues of dairy cows receiving endrin daily in their diet. J. Agr. & Food Chem. 6(7): 518-521.

68. Kingscote, A. A., and Jarvis, C. H. (1946) Report upon experiments conducted to establish the tolerance of turkeys to DDT. Canad. J. Comp. Med. 10(8): 211-218.

69. Kitselman, C. H., Dahm, P. A., and Borgman, A. R. (1950) Toxicologic studies of aldrin (Compound 118) on large animals. Amer. J. Vet. Res. 11(41): 378-381.

70. Knipling, E. F. (1953) The greater hazard-insects or insecticides. J. Econ. Ent. 46(1): 1-7.

71. Konst, H., and Plummer, P. J. G. (1946) Studies on the toxicity of DDT for domestic and laboratory animals. Canad. J. Comp. Med. 10(5): 128-136.

72. Levine, N. D., Kantor, S., and Taylor, G. D. (1958) Trials of organic phosphorus nematocides in sheep and mice. Illinois Vet. 1: 6.

73. Marsden, S. J., and Bird, H. R. (1947) Effects of DDT on growing turkeys. Poultry Science 26(1): 3-6.

74. Marsh, Hadleigh, Johnson, L. H., Clark, R. S., and Pepper, J. H. (1951) Toxicity to cattle of toxaphene and chlordane grasshopper baits. Mont. Agr. Exp. Sta. Bull. 477, 11 pp.

75. McGregor, W. S., Radeleff, R. D., Claborn, H. V., and Bushland, R. C. (1955) Dieldrin, aldrin and lindane as systemic insecticides against livestock pests. Agr. Chem. 10(1): 34-35, 123.

76. Metcalf, R. L., Fukuto, T. R., Reynolds, H. T., and March, R. B. (1955) Schradan residues in cotton and cottonseed products. J. Agr. & Food Chem. 3(1): 1011-1013.

77. Mitchell, J. W., Hodgson, R. C., and Gaetjens, C. E. (1946) Tolerance of farm animals to feed contining 2,4-dichlorophenoxyacetic acid. J. Animal Science 5: 226-232.

78. Moore, E. N., and Carter, R. D. (1954) Toxicity of chlordane to turkey poults. Poultry Science 33: 654-655.

79. Nickerson, W. J., and Radeleff, R. D. (1951) Effects of inhalation of chlordane vapors upon young chickens. Vet. Med. 46(8): 314, 326.

80. Orr, L. W., and Mott, L. O. (1945) The effects of DDT administered orally to cows, horses, and sheep. J. Econ. Ent. 38: 428-52.

81. Plapp, F. W., and Casida, J. E. (1958) Bovine metabolism of organophosphorus insecticides. Metabolic fate of 0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate in rats and a cow. J. Agr. & Food Chem. 6(9): 662-666.

82. Pfadt, R. E., and Ryff, J. F. (1955) Safe use of dieldrin dust for sheep ked control. J. Econ Ent. 48(2): 195-198.

83. Radeleff, R. D. (1948) Chlordane poisoning: symptomatology and pathology. Vet. Med. 43(8): 342-347.

84. Radeleff, R. D. (1949) Toxaphene poisoning: symptomatology and pathology. Vet Med. 44(10): 436-442.

85. Radeleff, R. D. (1951) Effects of various levels of lindane in the feed of beef cattle. Vet. Med. 46(3): 105-106, 119.

86. Radeleff, R. D., and Bushland, R. C. (1950) Acute toxicity of chlorinated insecticides applied to livestock. J. Econ. Ent. 43(3): 358-364.

87. Radeleff, R. D., Claborn, H. V., Wells, R. W., and Bushland, R. C. (1951) Toxaphene residues in fat of sprayed cattle. Vet. Med. 46(8): 305-308.

88. Radeleff, R. D., Claborn, H. V., Wells, R. W., and Nickerson, W. J. (1952) Effects on beef cattle of prolonged treatment with a DDT spray. Vet. Med. 47(3): 94-96.

89. Radeleff, R. D., and Bushland, R. C. (1953) Benzene hexachloride poisoning of emaciated sheep. Vet. Med. 48(2): 53-58.

90. Radeleff, R. D., Woodard, G. T., Nickerson, W. J., and Bushland, R. C. (1955) The acute toxicity of chlorinated hydrocarbon and organic phosphorus insecticides to livestock. U. S. Dept. Agr. Tech. Bull. 1122.

91. Radeleff, R. D., and Woodard, G. T. (1956) Toxicological problems in the use of systemic insecticides for livestock. J. Econ. Ent. 49(1): 89-91.

92. Radeleff, R. D., and Woodard, G. T. (1956) Cholinesterase activity of normal blood of cattle and sheep. Vet. Med. 51(11): 512-514.

93. Radeleff, R. D., and Woodard, G. T. (1956) The past, present, and future of systemic parasiticides. Vet. Med. 51(4): 152-154.

94. Radeleff, R. D. (1956) Hazards to livestock of insecticides used in mosquito control. Mosquito News 16(2): 79-80.

95. Radeleff, R. D., and Woodard G. T. (1956) The diagnosis and treatment of chemical poisoning of animals with particular reference to insecticides. Proc. Book, Am. Vet. Med. Assoc. 92: 109-113.

96. Radeleff, R. D., and Woodard, G. T. (1957) The toxicity of organic phosphorus insecticides to livestock. J.A.V.M.A. 130(5): 215-216.

97. Radeleff, R. D., and Woodard, G. T. (1957) Toxicological studies of Dow ET-57 in cattle and sheep. J. Econ. Ent. 50(3): 249-251.

98. Radeleff, R. D., Woodard, G. T., and Bushland, R. C. (1956) Toxicity of insecticides. USDA Yearbook of Agriculture, pp. 131-142.

99. Radeleff, R. D. (1958) The toxicity of insecticides and herbicides to livestock. Adv. in Vet. Sci. 4, 265-276, Academic Press.

100. Radeleff, R. D., and Woodard, G. T. (1956(1958)) The toxicity of systemically active insecticides administered to livestock. Proc. Tenth Inter. Cong. of Ent. 3: 737-740.

101. Rieck, R. F., and Keith, R. K. (1958) Studies on anthelmintics for cattle. IV The organic phosphorus compound 0,0-dimethyl 2,2,2-trichloro-1-hydroxymethyl phosphonate (Bayer L 13/59). Aust. Vet. Jour. 34: 93.

102. Robbins, W. E., Hopkins, T. L., and Eddy, G. W. (1957) Metabolism and excretion of phosphorus-32 labeled diazinon in a cow. J. Agr. & Food Chem. 5(7): 509-513.

103. Roberts, R. H., Radeleff, R. D., and Kaplanis, J. N. (1958) Bioassay of the blood from cattle treated with American Cyanamid 12880. J. Econ. Ent. 51(6): 861-864.

104. Rosenberg, M. M., and Adler, H. E. (1950) Comparative toxicity of DDT and chlordane to young chicks. Amer. J. Vet. Res. 11(38): 142-144.

105. Rosenberg, M. M., and Tanaka, T. (1950) Toxicity of chlordane to growing chickens, Amer. J. Vet. Res. 11(40): 233-235.

106. Rosenberg, M. M., Tanaka, T., and Adler, H. E. (1950) Toxicity of chlordane to laying pullets. Amer. J. Vet. Res. 11(40): 236-239.

107. Rowe, V. K. (1952) Health hazards associated with handling and use of herbicides. Agr. Chem. 7(2): 42-45, 110-113.

108. Rowe, V. K., and Hymas, T. A. (1954) Summary of toxicological information on 2,4-D and 2,4,5-T type herbicides and an evaluation of the hazards to livestock associated with their use. Amer. J. Vet. Res. 25(57): 622-629.

109. Sherman, M., and Rosenberg, M. M. (1953) Acute toxicity of four chlorinated dimethanonaphthalene insecticides to chicks. J. Econ. Ent. 46(6): 1067-1070.

110. Sherman, M., and Rosenberg, M. M. (1954) Subchronic toxicity of four chlorinated dimethanonaphthalene insecticides to chicks. J. Econ. Ent. 47(6): 1082-1083.

111. Smith, R. F., Hoskins, W. M., Fullmer, O. H. (1948) Secretion of DDT in milk of dairy cows fed low residue alfalfa hay. J. Econ. Ent. 41(5): 759-762.

112. Sparr, B. I., Clark, V. C., Vallier, E. E., and Baumhover, A. H. (1952) Studies on stability of toxaphene emulsions in dipping vats. U. S. Bur. Ent. & Plant Quar. E-849, 11 pp. illus.

113. Spicer, S. S., Sweeney, T. R., Von Oettingen, W. F., Lillie, R. D., and Neal, P. A. (1947) Toxicological observations on goats fed large doses of DDT. Vet. Med. 42(8): 289-293.

114. Stoddard, G. E., Shupe, J. L., Bateman, G. Q., Harris, J. R., Harris, L. E., and Greenwood, D. A. (1957) Effects of feeding aldrin- and chlordane-treated alfalfa hay and of aldrin in corn oil to lactating dairy cows. Abstr. papers, 132nd meeting Amer. Chem. Soc., 14 A-15 A.

115. Telford, H. S., and Harwood, P. D. (1945) DDT fails to remove horse bots. J. Econ. Ent. 38: 399.

116. Telford, H. S., and Guthrie, J. E. (1945) Transmission of the toxicity of DDT through the milk of white rats and goats. Science 102(2660): 647.

117. Terriere, L. C., and Ingalsbe, D. W. (1953) Translocation and residual action of soil insecticides. J. Econ. Ent. 46(5): 751-753.

118. Terriere, L. C., Kiigemagi, Ulo, and England, D. C. (1959) Endrin content of body tissues of steers, lambs and hogs receiving endrin in their daily diet. J. Agr. & Food Chem. 6(7): 516-518.

119. Turner, H. F., and Eden, W. G. (1952) Toxicity of chlordane to chickens. J. Econ. Ent. 45(1): 130.

120. Walker, Kenneth C., Goette, Mary B., and Batchelor, Gordon S. (1954) Dichlorodiphenyltrichlorethane and dichlorodiphenyldichloroethane content of prepared meals. J. Agr. & Food Chem. 2(20): 1034-1037.

121. Welch, Howard (1948) Tests of the toxicity to sheep and cattle of certain of the newer insecticides. J. Econ. Ent. 41(1): 36-39.

122. Wingo, C. W., and Crisler, O. S. (1948) Effect of DDT on dairy cattle and milk. J. Econ. Ent. 41(1): 105-106.

SYSTEMIC INSECTICIDES IN ANIMALS

Frederick W. Plapp, Jr., William F. Chamberlain, and R. D. Radeleff[1]

Recent research has demonstrated the efficiency of a number of the new agricultural chemicals in controlling pests that affect our crops and animals. At the same time these findings have opened new fields of study, for knowledge as to the effectiveness of a pesticide is not enough. We must know whether it leaves any toxic residues in the crop or animal where it is used. Such studies must be conducted for several reasons. Aside from strictly a research standpoint, data must be obtained to meet the requirements of various State and Federal agencies, especially those responsible for the jurisdiction of Public Law 518, the Miller Amendment to the Federal Food, Drug, and Cosmetic Act.

In working with systemic insecticides, as well as with other agricultural chemicals, the researcher should know what happens to them. In order to measure the residues present, he must determine the nature of the metabolism of the insecticide within the animal. He must know whether the chemical is converted to other toxic materials. He must ascertain the nature of the detoxication processes that take place--how the insect and the mammal defend themselves against the insecticide. Studies on the metabolism of insecticides in animals are therefore aimed at finding out both what the animal does to the material and what the material does to the animal.

Much of our present knowledge of the metabolism of insecticides has been gained through the study of compounds that show systemic activity. Utilizing techniques developed in conjunction with metabolism studies in animals, information has been gained as to the reasons why some insecticides are more toxic to certain insects than to other insects or to mammals. These techniques have also been used in investigations on resistance.

It is now known that a number of insect pests of animals can be controlled with systemic insecticides. Interest in this field has centered on the cattle grub, a parasite of major importance to the cattle industry. Estimates of the annual loss due to this parasite have been reported as $100 million a year (Haeussler 1952). Recent reviews summarized progress in cattle grub control through 1958 (Graham 1959, Peterson and Kingscote 1959).

Some of the earlier research in this field was done on chlorinated hydrocarbons, such as DDT, lindane, and dieldrin. Some of them were found to show systemic activity against cattle grubs (McGregor, et al. 1955). However, it has been found that compounds of this class tend to be stored in fat for long periods of time and secreted in milk of treated animals. In recent years recommendations pertaining to their use on livestock and poultry have been either withdrawn or restricted, owing to the problem of residues.

[1] Entomologist, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Corvallis, Oreg.; Entomologist, Entomology Research Division; and Veterinarian in Charge, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Kerrville, Tex., respectively.

Work on the metabolism of these compounds has been limited. It has been established that aldrin is converted to dieldrin in the animal body but dieldrin is apparently not metabolized (Bann, et al. 1956). In mice lindane is rapidly broken down to a number of products, but they have not been identified (Van Asperen and Oppenoorth 1954). In insects a dehydrochlorinated derivative of lindane, pentachlorocyclohexene, has been isolated (Sternburg and Kearns 1956).

The problems associated with the use of chlorinated hydrocarbon insecticides have caused a shift of interest to the organophosphorus insecticides, and by far the greatest amount of work on systemic materials has been done with this class of compounds. There are several reasons for this. Although some of them are highly toxic, others, contrary to popular belief, are among the safest insecticides available. Very favorable toxicity ratios occur; for instance, ronnel is about 1,000 times more toxic to houseflies than to mammals (O'Brien 1959). Moreover, most of them are rapidly metabolized and eliminated in mammals.

Although several compounds in this group have been found to be active as systemics against various insect pests, only two, ronnel and Co-Ral, are currently recommended. Both are used against cattle grubs, ronnel being administered orally as a bolus and Co-Ral applied in spray form. Several other promising materials are under investigation, and it is almost certain that better and safer ones will be found.

This paper consists of a review of the methods used in studying the metabolism and determining the residues associated with the administration of systemic insecticides to animals. Such information forms the basis for recommending the way they may be used without leaving residues in meat or milk, or for establishing the tolerances needed, in the event a tolerance is permitted. In addition, a more detailed account will be given on the metabolism of the two recommended insecticides and some of those being evaluated at the present time.

METHODS USED

Use of Radioactive Insecticides.--Many of the studies on the metabolism of organophosphorus compounds in animals have utilized radioactive samples of the test insecticide. Phosphorus-32 has usually been the isotope used, although some materials have been synthesized with carbon or sulfur labels. It is doubtful that the vast amount of information accumulated could have been obtained without the use of labeled tracers, which allow for extremely sensitive, accurate, and rapid methods of analysis.

Animals are sprayed or treated orally or intramuscularly with labeled samples of the test materials as far as possible by the methods previously found effective in field tests. Following treatment, animals are held in metabolism stalls to facilitate the collection of the many samples required. Excretory samples must be collected quantitatively and numerous blood samples taken. Other samples, such as milk, hair, and fat, may be needed.

Toxicity to Mammals.--The first study is that of toxicity. Organophosphorus compounds are toxic to mammals because of their ability to inhibit cholinesterase (ChE), an enzyme involved in controlling transmission of nerve impulses. The percent of normal ChE activity in the blood of treated animals usually reflects the amount of poisoning that has occurred. Determinations may be made by manometric, colorimetric, or electrometric methods. The enzyme is progressively inhibited by increasing dosages, but the degree of inhibition does not indicate the presence or absence of symptoms. Indeed, many animals die with 50 percent or more of the activity remaining in the blood, whereas others exist comfortably with no detectable activity in the blood. Results obtained with several insecticides are presented in table 1.

Blood.--Information on the metabolism of the insecticide itself can also be gained by studying blood samples from treated animals. Determinations of total radioactivity

TABLE 1.--Maximum cholinesterase inhibition due to administration of several systemic insecticides to cattle.

Insecticide (mg./kg.)	Method of treatment	Maximum ChE inhibition	Reference
		Percent	
Ronnel:			
100	Oral	55	21
100	do.	40	24
Co-Ral:			
40	Dermal	40	14
52	do.	42	25
37	do.	36	25
Dimethoate:			
10	Oral	31,69	13
10	Intramuscular	42	13
9, 10	Oral	40	4
40	do.	80	4
Ruelene:			
11	Intramuscular	24	22
40	Dermal	25	6

present in blood provide information as to how fast the insecticide and its metabolites are absorbed by the animal. In addition, the radioactivity can be fractionated into insecticide, oxidation products, and hydrolysis products to find out how long the unchanged compound is present, what levels it reaches, and when all the insecticide is gone. The rate at which these changes occur is related to the solubility of the compound and to the ease with which it is detoxified by the animal.

Further information can be gained by bioassaying samples of the blood. The blood of treated animals may be fed to stableflies and the toxicity compared with the results obtained by radiometric analyses (Kaplanis, et al. 1956). The use of bioassay techniques on blood is especially valuable in determining the possibility that the animal might convert the compound to a more toxic material (Roberts, et al. 1958). The formation of such a material--for example, the oxygen analog of a phosphorothioate insecticide--has been demonstrated with several systemics. Data on peak levels of several insecticides in the blood of treated animals are summarized in table 2.

Milk.--An important consideration in the development of systemic insecticides is the nature of residues in the milk of treated animals. Compounds that are not secreted in milk could be used to treat lactating dairy animals. All the systemic insecticides tested thus far on dairy animals at dosages that control cattle grubs have been found in milk samples taken shortly after treatment. Because of this finding, no systemic insecticide is presently recommended for use on lactating dairy animals. Data on residues in milk are summarized in table 3.

Excretory Products.--An insecticide fed to animals can either be stored as such or as an oxidized derivative, or it can be detoxified and excreted. It is now known that almost all the phosphorus insecticides are rapidly broken down and excreted by mammals.

Examination of excretory products of treated animals has shown the rate of excretion to vary greatly--from almost complete elimination within a few hours of treatment with some compounds to elimination over a period of weeks with others. This variation is affected by a number of factors, among which ease of absorption, solubility, susceptibility to hydrolysis, and route of administration are known to be important. Radioactivity in excretory products may reach very high levels.

TABLE 2.--Insecticide present in blood of cattle treated with several systemics.

Insecticide (mg./kg.)	Method of treatment	Peak level in blood		Reference
		Hours after treatment	p.p.m.	
Ronnel:				
100	Oral	8	1	21
100	do.	12	3.1	24
100	do.	8	1.4	11
Co-Ral:				
40	Dermal	120	.015	14
37-52	do.	-	<.035	25
20	Oral	12	.02	12
Dimethoate:				
9,10	Oral	2	1.5	4
10	do.	3-6	1-2.3	13
10	Intramuscular	1	4.9	13
Ruelene:				
11	Intramuscular	2	.5	22
40	Dermal	4	1.8	6

TABLE 3.--Insecticide residues in milk of dairy cows treated with systemic insecticides.

Insecticide (mg./kg.)	Method of treatment	Maximum p.p.m.	Reference
Ronnel			
100	Oral	32	21
Co-Ral			
40	Dermal	.06	14
Dimethoate			
9	Oral	.4	4
40	do.	4	4

Information on the excretion of several organophosphorus insecticides is summarized in table 4. The data indicate that the urine is usually the major route of elimination. Only water-soluble compounds can be excreted by this route and most of these insecticides are not sufficiently soluble to be excreted directly. Most of the radioactivity present in urine samples represents detoxication products of the insecticides. From studies of the breakdown products present in the urine has come much basic information on the metabolism of insecticides, which will be discussed in more detail with the individual chemicals.

Excretion in the feces is usually less important, although with some compounds administered orally it may represent a considerable fraction of the total. The radioactivity in the feces may be in the form of both insecticide and detoxication products. Occasionally enough insecticide is excreted here to be toxic to feces-breeding flies.

TABLE 4.--Excretion of several organophosphorus insecticides by cattle.

Insecticide (mg./kg.)	Method of treatment	Percent of dosage recovered in -		Hours after treatment for peak excretion		Reference
		Urine	Feces	Urine	Feces	
Ronnel:						
100	Oral	>49	5	18-32	24	21
100	do.	86	7	18-24	12-18	24
100	do.	74	2	4-8	30-36	11
Co-Ral:						
37-52	Dermal	3-6	-	120	-	25
27-65	do.	1-3	-	0-48	-	13
10-20	Oral	35-38	34-36	10-12	36	13
Dimethoate:						
9-10	Oral	72	4	-	-	4
10	do.	91-95	4-5	3-6	6-30	13
10	Intramuscular	97	1	1-3	9-12	13
Ruelene:						
11	Intramuscular	35	12	12-18	24-30	22
40	Dermal	12	trace	8-12	8-12	6

Residues.--After 1 to 6 weeks the treated animal is slaughtered, and tissue samples are taken for analysis. If any residues are present, their nature must be determined. The time after treatment before meat of treated animals is free of insecticide and safe for human consumption must also be determined. Samples of edible muscles are taken as well as of fat where insecticide could be stored. Many of the organs--such as the liver, lung, kidney, and brain--are sampled in further attempts to learn more about what happened to the insecticide.

Total residues are determined in all tissues sampled, based on radioactivity. After total residues have been measured, their nature must be determined. Data on residues will be presented in the discussion of individual compounds.

FATE OF INDIVIDUAL INSECTICIDES

Ronnel.--Ronnel (O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothiate), also known as Korlan, Dow ET-57, and Trolene, was the first organophosphorus insecticide to be approved as an animal systemic. In studies at Corvallis, Oreg., and Kerrville, Tex., in 1955 it was observed to be effective against grubs when administered orally to cattle (Roth and Eddy 1956, McGregor and Bushland 1956). A dosage of 110 mg./kg. is recommended for grub control. The compound is now registered for use on beef animals, a 60-day period between the time of administration and slaughter being required. Toxicological studies on cattle, sheep, and laboratory animals have been reported (Radeleff and Woodard 1957, McCollister, et al. 1959).

The metabolism of P^{32}-labeled ronnel in cattle has been investigated by several workers. At Kerrville metabolism and excretion in a Hereford heifer and its systemic action on screwworms and stableflies have been studied (Kaplanis, et al. 1956). Peak levels of the insecticide occurred in the blood after 8 hours and the amount was closely correlated with toxicity to both insects. Analyses of the blood showed both ronnel and its oxygen analog, but both were dissipated within 60 hours. Of the administered insecticide, 74 percent was eliminated in the urine and 2 percent in the feces within 3 days.

At Corvallis, Robbins, et al. (1956) studied the fate of the labeled compound in a Guernsey bull calf. Their data on levels in the blood were similar to those reported from Kerrville. Cholinesterase in the treated animal was about 40 percent inhibited and was recovering 2 weeks after treatment.

Near quantitative recovery of the administered radioactivity in the urine and feces was found by 11 days after treatment. Small quantities of unmetabolized insecticide were excreted in the feces, but none in the urine.

After 14 days the animal was sacrificed and tissue samples were taken for analysis. Total radioactivity was highest in the brain, liver, and kidney, ranging from 9 to 12 p.p.m., but only small amounts of the radioactive materials partitioned as unmetabolized insecticide. Total residues in meat were very low, and less than 0.05 p.p.m. was insecticide. Fat samples, however, still contained 7 p.p.m. after 2 weeks.

In studies at the University of Wisconsin Plapp and Casida (1958) found the rates of metabolism in the blood, urine, and feces of a lactating Holstein cow to be similar to those reported from Kerrville and Corvallis. In milk taken from the animal 8 hours after treatment 32 p.p.m. of the insecticide were present. The amount declined rapidly, but 0.4 p.p.m. was still present after a week. Of the total dose 2 percent was secreted in the milk.

After 7 days the cow was sacrificed and tissue samples were taken for analysis. As much as 18 p.p.m. of hexane-soluble radioactivity was found in fat samples, but it was not determined whether it was all insecticide or included other materials of similar solubility.

The nature of ronnel metabolism was further studied in white rats and houseflies (Plapp and Casida, 1958). Since paper chromatographic techniques available at the time were unsatisfactory for separating the possible hydrolytic metabolites, an ion-exchange chromatographic separation was developed. It was demonstrated that in mammals ronnel was subject to hydrolysis at both the P-O-phenyl and P-O-methyl bonds whereas in insects hydrolysis occurred largely at the P-O-phenyl bond. The characterization of hydrolytic metabolites formed by hydrolysis of the P-O-methyl bond was the first report on compounds of this type. The amount of metabolites produced by hydrolysis of the P-O-methyl bond was much higher in mammals than in insects and it was postulated that this difference may account, at least in part, for the lower toxicity of ronnel to mammals than to insects.

Co-Ral.--The second insecticide to be registered for use as an animal systemic was Co-Ral (O-(3-chloro-4-methylumbelliferone) O,O-diethyl phosphorothioate), also known as Bayer 21/199. Preliminary tests with this compound showed that it was only partially effective as a systemic when administered orally to cattle (McGregor, et al. 1954), but later it was found to be highly active as a spray (Brundrett, et al. 1957). It is currently recommended as a 0.5 percent spray at the rate of 1 gallon per animal. A minimum of 45 days is required between treatment and slaughter.

The metabolism and residues associated with the administration of Co-Ral to cattle have been extensively studied (Kaplanis, et al. 1959, Krueger, et al. 1959, Robbins, et al. 1959). When applied as a spray, most of the insecticide remained on the hair and hide. Levels in the blood were very low, reaching a maximum of 0.27 p.p.m. in the different studies. Maximum ChE depression to about 60 percent of normal occurred 1 week after treatment. Residues in milk were never higher than 0.1 p.p.m. Only small fractions of the dose were excreted in the urine and feces.

When administered orally, as much radioactivity was excreted in the feces as in the urine (34 percent). Large amounts of the material in the feces were unmetabolized insecticide, indicating that it was poorly absorbed from the digestive tract.

In an animal sacrificed 2 weeks after spraying at 52 mg./kg. the most insecticide in any tissue was 0.11 p.p.m. in subcutaneous fat. In a dairy animal sacrificed 6 weeks after dermal treatment at 40 p.p.m., residues in all internal tissues were less than 0.06 p.p.m. However, high residues of unmetabolized insecticide were still present on the hide several weeks after treatment. A sensitive chromatographic technique utilizing alumina was developed which allows for rapid separation of Co-Ral, its oxygen analog, and labeled phospholipids with similar partitioning properties (Krueger, et al. 1959).

The peculiar property of Co-Ral--poorly effective orally, but systematically active dermally--may be ascribed to its chemical structure. Krueger et al. demonstrated that the pyrone ring can be opened to form a water-soluble derivative under alkaline conditions. When acidified, the ring is reclosed, and so the chemical can be converted to a water-soluble material without losing its toxicity. The same property has recently been made the basis for several methods of determining Co-Ral residues (Claborn, et al. 1960, Kane, et al. 1960). Ion-exchange chromatography has demonstrated that Co-Ral, like ronnel, is detoxified by hydrolysis of both the P-O-phenyl and P-O-alkyl groupings. There is evidence that the process goes completely to phosphoric acid. In rats differences in the route of administration have resulted in differences in the site of hydrolytic attack.

Dimethoate.--Dimethoate (0,0-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate) is another organic phosphorus insecticide that has shown promise as an animal systemic. Studies on its toxicology (Hewitt, et al. 1958) and also metabolism and residues associated with its oral and intramuscular administration to cattle have been reported (Dauterman, et al. 1959, Kaplanis, et al. 1959).

Oral administration of dimethoate to cattle at 10 mg./kg. caused 30 to 40 percent inhibition of RBC ChE, and administration at 40 mg./kg. caused an 80 percent reduction in ChE activity and symptoms of poisoning were noted. Highest levels of insecticide in the blood occurred 2 to 8 hours after oral treatment and within 1 hour following intramuscular injection. Within 24 hours substantially all insecticide had disappeared from the blood. Paper chromatographic evidence was obtained for the conversion of dimethoate to its oxygen analog and for the occurrence of both materials in blood.

In bioassay studies on the blood of animals treated with the radioactive dimethoate, Roberts, et al. (1958) demonstrated the occurrence of a metabolite more toxic to stableflies than the parent compound. The concentration of the metabolite increased as the concentration of the insecticide declined, but both were completely dissipated within 24 hours.

Dimethoate is the most rapidly metabolized and eliminated compound yet studied. About 90 percent of the administered material was accounted for in the urine within 24 hours of oral and 9 hours of intramuscular treatment. Only trace amounts were eliminated in the feces.

A total of 1.5 p.p.m. of dimethoate plus metabolites was present in the milk of a cow 8 hours after oral treatment at 9 mg./kg., and it declined to less than 0.02 p.p.m. after 48 hours. With a cow treated at 40 mg./kg. 4.0 p.p.m. was present in the 0-8 hour milk sample, and within 48 hours there was less than 0.04 p.p.m.

Dimethoate leaves almost no residues in tissues of treated animals. Fat biopsies showed that 3 p.p.m. was present after 3 hours, and less than 0.1 p.p.m. after 8 hours. In animals treated orally at 10 mg./kg. there was less than 0.1 p.p.m. in all tissues within 14 days.

The metabolism of dimethoate in cattle has been shown to be complex by both paper and ion-exchange chromatographic techniques. Kaplanis, et al. (1959) developed an acetonitrile:water system that gave excellent separation of a large series of hydrolytic metabolites. They found that most of the radioactivity in the urine was an unknown ionized

compound partially extractable from acidified urine with chloroform. The paper-chromatographic technique developed in that study has found wide application in studies of the metabolism of other organophosphorus insecticides.

Using ion-exchange chromatography, Dauterman, et al. (1959) also separated a large series of dimethoate metabolites. They showed that the major unknown metabolite was the carboxylic acid derivative formed by hydrolysis at the carbamoyl bond. They further showed that in both cattle and white rats the insecticide was detoxified by hydrolysis at all the possible esteratic sites.

Ruelene.--Another compound to undergo recent extensive testing as an animal systemic is Ruelene (4-tert-butyl-2-chlorophenyl methyl methylphosphoramidate). This insecticide is effective against cattle grubs either orally or as a spray. Preliminary data on its effectiveness and toxicology have been published (Dow ACD Bulletin 1959).

The metabolism of Ruelene has been investigated at Kerrville, Tex., and Corvallis, Oreg., following dermal and intramuscular administration to cattle. Studies thus far have shown that the insecticide is absorbed most rapidly following intramuscular treatment, peak levels in the blood occurring after 6 hours and after 12 hours following dermal treatment.

Excretion of hydrolytic metabolites of the insecticides occurs fairly slowly and is incomplete. With orally and intramuscularly treated animals 35 to 42 percent of the dose was recovered in the urine and feces within 2 weeks. Following dermal administration recovery was considerably lower.

The poor recovery of metabolites of Ruelene in the excretory products indicated that large amounts of the insecticide might remain in treated animals. Upon sacrifice, the total radioactivity was high in a number of tissues. However, fractionation showed most of this to be phosphoric acid and that there was almost no unmetabolized insecticide, residues being less than 0.1 p.p.m. It is therefore apparent that the metabolism of the insecticide goes completely to phosphoric acid and that the phosphate so produced is incorporated into the normal phosphorus metabolism of the animal.

That this metabolism is complex and complete has been indicated by paper and ion-exchange chromatographic studies. From 7 to 11 metabolites have been observed. At present, however, they are incompletely characterized, and it is not possible to outline a metabolic pathway for the insecticide.

CONCLUSIONS

Many facets of the problems involved in the use of systemic insecticides in animals need further study. Among the most urgent is the treatment of dairy animals. All the compounds studied thus far which show systemic activity have been secreted in the milk, although often at very low levels. Residues in milk have been much lower in animals treated dermally than in those treated orally. However, with dermal treatments there is the problem of continuous slow absorption and the possibility that trace amounts of insecticide might be present for some time.

The data available show that a high degree of cattle grub control can be obtained with levels of insecticide that leave no toxic residues present in edible tissues. Control can be obtained with both oral and dermal treatments and, possibly, by intramuscular injection.

Much of our present knowledge of the metabolism of organic-phosphorus insecticides has been gained through studies of systemics. Techniques developed in these studies have found wide applications in investigation of such problems as the metabolism of insecticides in resistant and susceptible strains of insects. From these studies has come a considerable understanding of the fate of insecticides in both insects and mammals.

REFERENCES CITED

1. Bann, J. M., T. J. DeCino, N. W. Earle, and Y. P. Sun. 1956. The fate of aldrin and dieldrin in the animal body. Jour. Agric. Food Chem. 4(11): 937-41.

2. Brundrett, H. M., W. S. McGregor, and R. C. Bushland. 1957. Systemic cattle grub control with Bayer 21/199 sprays. Agric. Chem. 12(6): 36-7, 123, 125.

3. Claborn, H. V., M. C. Ivey, and H. D. Mann. 1960. A colorimetric method for the determination of Co-Ral in animal tissues. Jour. Econ. Ent. 53(2): 263-5.

4. Dauterman, W. C., J. E. Casida, J. B. Knaak, and T. Kowalczyk. 1959. Bovine metabolism of organophosphorus insecticides. Metabolism and residues associated with oral administration of dimethoate to rats and three lactating cows. Jour. Agric. Food Chem. 7(3): 188-93.

5. Dow Chemical Company. 1959. Ruelene a new anthelmintic and systemic insecticide. ACD Information Bull. 115. Midland, Mich.

6. Gatterdam, P. E., W. F. Chamberlain, D. E. Hopkins, M. A. Gregg, and J. J. Matter. 1959. Studies with P^{32} labeled Ruelene in cattle, sheep and guinea pigs. Unpublished report, U.S.D.A., A.R.S., Ent. Res. Div., Kerrville, Tex.

7. Graham, O. H. 1959. Cattle grub control with systemic insecticides. Sw. Vet. 12(4): 277-80.

8. Haeussler, G. J. 1952. Losses caused by insects. U.S. Dept. Agric. Yearbook (Insects), pp. 141-6.

9. Hewitt, R., J. Emor, J. Entwhistle, J. Pankavich, R. Thorson, W. Wallace, and E. Waletzky. 1958. Carbamoyle alkyl phosphorodithioates as chemotherapeutic agents: Effects of Dimethoate against grubs in cattle. Jour. Econ. Ent. 51(4): 445-50.

10. Kane, R. F., C. J. Cohen, W. R. Betker, and D. MacDougall. 1960. Assay of Co-Ral in technical material and formulated products. Jour. Agric. Food Chem. 8(1): 26-9.

11. Kaplanis, J. N., D. E. Hopkins, and C. B. Cowan. 1956. Studies with P^{32} labeled ET-57. Unpublished report, U.S.D.A., A.R.S., Ent. Res. Div., Kerrville, Tex.

12. Kaplanis, J. N., D. E. Hopkins, and G. H. Treiber. 1959. Dermal and oral treatments of cattle with phosphorus-32-labeled Co-Ral. Jour. Agric. Food Chem. 7(7): 483-6.

13. Kaplanis, J. N., W. E. Robbins, D. I. Darrow, D. E. Hopkins, R. E. Monroe, and G. Treiber. 1959. The metabolism of dimethoate in cattle. Jour. Econ. Ent. 52(6): 1190-94.

14. Krueger, H. R., J. E. Casida, and R. P. Niedermeier. 1959. Bovine metabolism of organophosphorus insecticides. Metabolism and residues associated with dermal application of Co-Ral to rats, a goat, and a cow. Jour. Agric. Food Chem. 7(3): 182-8.

15. McCollister, D. D., F. Oyen, and V. K. Rowe. 1959. Toxicological studies of 0,0-dimethyl-0-(2,4,5-trichlorophenyl) phosphorothioate (Ronnel) in laboratory animals. Jour. Agric. Food Chem. 7(10): 689-93.

16. McGregor, W. S., R. D. Radeleff, and R. C. Bushland. 1954. Some phosphorus compounds as systemic insecticides against cattle grubs. Jour. Econ. Ent. 47(3): 465-7.

17. McGregor, W. S., R. D. Radeleff, R. C. Bushland, and H. V. Claborn. 1955. Dieldrin, aldrin, and lindane as systemic insecticides against cattle grubs and screwworms. Agric. Chem. 10(1): 34-6, 123.

18. McGregor, W. S., and R. C. Bushland. 1957. Tests with Dow ET-57 against two species of cattle grubs. Jour. Econ. Ent. 50(3): 246-9.

19. O'Brien, R. D. 1959. Comparative toxicology of some organophosphorus compounds in insects and mammals. Can. J. Biochem. Physiol. 37(9): 1113-22.

20. Peterson, D. G., and A. A. Kingscote. 1959. Control of animal parasites with systemic insecticides. Can. J. Biochem. Physiol. 37(9): 1105-12.

21. Plapp, F. W., and J. E. Casida. 1958. Bovine metabolism of organophosphorus insecticides. Metabolic fate of 0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate in rats and a cow. Jour. Agric. Food Chem. 6(9): 662-7.

22. Plapp, F. W., W. S. Bigley, and D. I. Darrow. 1959. Studies on the metabolism of P^{32}-Ruelene in a Hereford steer. Unpublished report. U.S.D.A., A.R.S. Ent. Res. Div., Corvallis, Oreg.

23. Radeleff, R. D., and G. T. Woodard. 1957. Toxicological studies of Dow ET-57 in cattle and sheep. Jour. Econ. Ent. 50(3): 249-51.

24. Robbins, W. E., T. L. Hopkins, and G. W. Eddy. 1956. The fate of P^{32}-labeled Dow ET-57 in the bovine body. Unpublished report. U.S.D.A., A.R.S., Ent. Res. Div. Corvallis, Oreg.

25. Robbins, W. E., T. L. Hopkins, D. I. Darrow, and G. W. Eddy. 1959. Studies with P^{32}-Bayer 21/199 sprayed on cattle. Jour. Econ. Ent. 52(2): 214-17.

26. Roberts, R. H., R. D. Radeleff, and J. N. Kaplanis. 1958. Bioassay of the blood from cattle treated with American Cyanamid 12,880. Jour. Econ. Ent. 51(6): 861-4.

27. Roth, A. R., and G. W. Eddy. 1957. Tests with Dow ET-57 against cattle grubs in Oregon. Jour. Econ. Ent. 50(3): 244-6.

28. Sternburg, J., and C. W. Kearns. 1956. Pentachlorocyclohexene, an intermediate in the metabolism of lindane by house flies. Jour. Econ. Ent. 49(4): 548-52.

29. Van Asperen, K., and F. J. Oppenoorth. 1954. Metabolism of gammabenzene hexachloride in the animal body. Nature 173: 1000.

HERBICIDES IN SOILS[1]

T. J. Sheets and L. L. Danielson[2]

INTRODUCTION

Many herbicides are applied directly to the soil surface as selective preemergence sprays and as nonselective soil sterilants. Other chemicals are applied subsurface or are thoroughly mixed with the soil after surface application. Residues remaining on leaves after foliar applications are carried to the soil in rainwater or fall to the soil when injured leaves abscise and fall. Therefore at least part of all herbicidal sprays eventually reach the soil.

Soils vary greatly in composition and reactivity. Many complex and ever-changing processes occur continuously in most soils. Soils are composed of mineral matter, organic matter, water, and air. The mineral fraction varies in amounts of sand, silt, and clay, and in types and amounts of clay minerals. The hydration and base saturation of the clay minerals also vary. The organic-matter fraction consists of decaying plant and animal residues and active soil flora and fauna. The organic and mineral colloids present in the soil contribute directly and indirectly to the extremely active nature of soil systems.

This mixture of mineral and organic matter is permeated by pore spaces of various sizes. These spaces are filled with water and air in a more or less reciprocal relation. The soil water contains many soluble compounds and serves as an essential medium for many physical and chemical processes. The soil atmosphere is composed of oxygen, carbon dioxide, nitrogen, and several minor gases. The composition of the soil atmosphere varies, particularly the oxygen and carbon dioxide contents. The complexity and variation of soil systems make the study of the fate of herbicides therein complicated and time consuming.

METHODS OF ASSAYING HERBICIDE RESIDUES IN SOILS

In most investigations on the persistence of herbicide in soils, researchers determine the presence of the active entity of the herbicide by growing sensitive plants. The influence of time on herbicidal residues has been measured by the growth of successive crops of test plants. This method, which has been used in both field and greenhouse experiments, is qualitative only.

Quantitative bioassays have been developed for some herbicides. Holstun and Loomis (35) measured the elongation of young shoots of germinated millet seeds to determine the concentration of the sodium salt of 2,2-dichloropropionic acid [dalapon] in soils. Burschel and Freed (11) used heights and weights of seedling oats to determine the concentrations of isopropyl N-phenylcarbamate [IPC], isopropyl N-(3-chlorophenyl) carbamate [CIPC], and 3-amino-1,2,4-triazole [amitrole] in soils. Rahn and Baynard (45) used weight of oat seedlings to assay quantitatively 3-(p-chlorophenyl)-1,1-dimethylurea [monuron] in soils. Biological assays developed for solutions and vapors of herbicides could be adapted for use with soils (17, 46, 55).

---

[1] A contribution from the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Mississippi Agricultural Experiment Station.

[2] Plant Physiologists, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Stoneville, Miss., and Beltsville, Md., respectively.

Some herbicides have been extracted from soils and their concentrations determined by physical or chemical methods. Methods are available for monuron, amitrole, CIPC, and pentachlorophenol [PCP] (10, 11, 30, 62). Whiteside and Alexander (61) followed the breakdown of several chlorinated phenoxy aliphatic acid herbicides in solutions inoculated with soil by the disappearance of the specific ultraviolet absorption.

A physical or chemical assay may be most suitable in one situation and a biological assay in another. Both types of analyses are useful in some cases. Rahn and Baynard (45) reported that the chemical method for the determination of monuron in soils was accurate if the assay was made within a few weeks after application. If soils were chemically assayed more than 1 month after treatment, values for monuron concentration were greater than those obtained by bioassay. Rahn and Baynard (45) suggested that this apparent disagreement could be explained since the chemical assay for monuron was based on p-chloroanaline, a nonphytotoxic, hydrolytic product of monuron.

FACTORS ALTERING HERBICIDES IN SOILS

Factors affecting the movement and persistence of herbicides in soils have been reviewed by several workers (1, 8, 25, 26, 32, 41, 43). Leaching, fixation by soil colloids, chemical and microbial decomposition, and volatilization were stressed in one or more of these papers. In this discussion microbial action, volatilization, adsorption, leaching, chemical reaction, photodecomposition, and absorption by plants will be considered for their significance in the performance and fate of soil-applied herbicides.

Microbial action.--Most organic herbicides subjected to appropriate tests have been inactivated more rapidly in soil under conditions favoring growth and proliferation of soil microorganisms. Absorption by microorganisms is one of the major pathways by which organic herbicides are detoxified. Perhaps 2-(2,4-dichlorophenoxy)propionic acid [2-(2,4-DP)], 2,4,5-trichlorophenoxyacetic acid [2,4,5-T], 2-(2,4,5-trichlorophenoxy) propionic acid [silvex], and 4-(2,4,5-trichlorophenoxy)butyric acid [4-(2,4,5-TB)] are exceptions (7, 61). Optimum oxygen, moisture, temperature, and nutrients favor microbial activity and also herbicidal detoxication. Numbers of soil microorganisms capable of inactivating 2,4-dichlorophenoxyacetic acid [2,4-D] apparently increase when 2,4-D is present in the soil (8, 42, 43, 61). Thus repeat applications of 2,4-D were less persistent in soil and therefore may be less effective herbicidally than the initial application. With the phenylureas and s-triazines such an increase in microbial activity apparently does not occur, because soils appear to exhibit about the same capacity for inactivation for long periods. Therefore it seems that with the phenylureas and the s-triazines the action of soil microorganisms is passive; organisms utilize them but not selectively or preferentially. Another explanation of this effect is that inactivation of these chemicals is catalyzed by heat-sensitive substances occurring in the soil as products of microbial activity and that the herbicides are not utilized directly by microorganisms as energy sources.

Bacteria, Bacterium globiforme and Flaviobacterium aquatile, which were capable of inactivating 2,4-D were isolated from soil and grown in pure culture (6, 8, 36). Evans and Smith (27) isolated a small, Gram-negative, motile soil organism which grew freely in a mineral-salt medium containing p-chlorophenoxyacetic acid as the only organic-carbon source. They separated 2-hydroxy-4-chlorophenoxyacetic acid and 4-chlorocatechol from the culture. The same investigators isolated a Gram-negative, motile rod which grew on a mineral-salt, 2,4-D medium. From this culture they separated a phenolic acid and presented evidence to suggest that the compound was 6-hydroxy-2,4-dichlorophenoxy-acetic acid. They hypothesized that hydroxylation of the ring was followed by ring cleavage.

Whiteside and Alexander (61) presented evidence suggesting that 4-(2,4-dichlorophenoxy)butyric acid [4-(2,4-DB)] was converted to 2,4-D by microorganisms in the soil and that a microflora capable of quickly inactivating both 2,4-D and 4-(2,4-DB) was present in soils which had received 4-(2,4-DB) previously.

Hill, et al. (33) reported that a soil bacterium of the Pseudomonas group was capable of oxidizing monuron particularly in the presence of yeast extract.

One group of herbicides, the esters of chlorophenoxy alcohols, becomes herbicidally active only on contact with the soil. In warm, moist soil sodium, 2,4-dichlorophenoxyethyl sulfate [sesone] is hydrolyzed to 2,4-dichlorophenoxyethanol in the presence of either microorganisms or acids (12, 13). The hydrolysis by microorganisms was attributed to acids secreted during their metabolism. The ethanol product is oxidized in the soil to 2,4-D, the active entity.

In experiments conducted by the senior author, 2-chloro-4,6-bis(diethylamino)-s-triazine [chlorazine] mixed in the soil became more toxic to seedling oats with time. This trend reversed after several months, the time depending on the soil type and concentration, and thereafter the herbicidal activity of cultures containing chlorazine decreased with time. The increase in toxicity could not be accounted for completely as a response to growing conditions in the greenhouse. Autoclaving the soil prior to treatment retarded the rate of onset of increased toxicity. If one ethyl group was lost from either or both of the amino substitutions, one or more compounds much more toxic than chlorazine would be formed. Perhaps formation of one or both of these compounds did occur in the soil.

The rates of inactivation of IPC, CIPC, and amitrole in the soil depended on the initial concentration of the herbicides; and the inactivation of these herbicides appeared to follow a first-order reaction (11). The rate of disappearance of monuron from soil was proportional to the concentration (33). Hill, et al. (33) concluded that although soil moisture and temperature often altered the rates of inactivation of monuron and 3-(3,4-dichlorophenyl)-1,1-dimethylurea [diuron], the first-order equation was probably applicable under usual field conditions. When monuron and diuron were applied at rates of 1 and 2 pounds per acre in more humid regions of the United States, major parts of the herbicides were inactivated each year. Accumulation from applications on the same soil 2 years in succession was negligible. Rahn and Baynard (45) found that monuron applied at 3.6 pounds per acre in two applications for 3 years in succession did not persist from one year to the next. When applied at 6.4 pounds per acre, monuron toxicity persisted from one year to the next, but no accumulation occurred. Research conducted in the arid Southwest during the last 7 years indicated that monuron and diuron did not accumulate significantly from successive annual applications at rates used for selective weed control in cotton. Some carryover often occurred, and the amount of carryover appeared to be related to weather conditions.

Current research indicates that the solvent used in the application of an herbicide may have a profound influence on the persistence of herbicidal activity (18). Ethel N,N-di-n-propylthiolcarbamate [EPTC] was applied in several solvents and incorporated into the soil. At weekly intervals up to 6 weeks after treatment, the soils were assayed by the use of oat plants as indicators of toxicity. When the commercial formulation of EPTC was applied in water, growth of oat plants seeded 6 weeks after treatment were markedly inhibited on flats which received 1 and 2 lb./A. The persistence of technical EPTC applied in acetone was comparable to that of the commercial formulation applied in water. However the rate of inactivation of technical EPTC applied in kerosene was much more rapid than that of the commercial formulation applied in water. Four weeks after treatment the 2 lb./A rate of technical EPTC applied in kerosene did not inhibit growth of oats.

Persistence of several groups of herbicides in the soil is related to halogenation of the benzene ring. This relation was demonstrated for certain chlorinated phenoxyacetic acids, carbamates, and phenylureas (21, 22, 23, 50). The results of Alexander and Aleem (3) indicated that resistance of chlorinated phenoxyalkyl carboxylic acid herbicides or their derivatives to microbial degradation was governed by the position of the halogen rather than by the number of halogens on the ring and that the linkage and type of aliphatic side chain also influenced susceptibility to microbial breakdown.

Volatilization.--All compounds are volatile to some degree. Volatility of some herbicides is very low and of little significance. However, measurable loss of others occurs from soil surfaces by vaporization.

The volatilities of formulations of the same basic herbicide structure may be quite different. The isopropyl ester of 2,4-D is more volatile than the octadecyl ester, which in turn is more volatile than the sodium salt (39). Many other esters and salts of 2,4-D exhibit various rates of vaporization.

Vapors of soil-applied herbicides have caused severe injury to treated crop plants in some instances. Vapors of 4,6-dinitro-o-sec-butylphenol [DNBP] after preemergence applications caused extensive injury to cotton in the Mississippi Delta in 1952 (25, 26, 34). DNBP injury was associated with high temperatures. Hollingsworth and Ennis (34) found that vapor injury to young cotton plants increased as soil moisture increased.

DNBP injury to cotton was reduced by application of lime and other basic materials to the treated soil surface (9, 19). Upon addition of a base, the phenol-phenate equilibrium was probably shifted to the phenate, which is less volatile than the phenol (9).

Volatilization of the carbamates has been related to their effectiveness as preemergence herbicides. IPC and CIPC volatilized rapidly from tinfoil and glass surfaces at high temperatures (4). IPC volatilized more rapidly than CIPC, particularly at temperatures of 60° to 85° F. The most volatile carbamates were found to be most phytotoxic (38); however, loss by volatility following preemergence application reduced the concentration of the more volatile compounds to nonherbicidal levels more rapidly than the less phytotoxic, less volatile compounds.

Many herbicides are formulated on granular carriers to reduce loss by volatility and leaching after application to the soil surface. The vapor and contact activities of CIPC-impregnated granular carriers were investigated by Danielson (17). The vapor activity of CIPC was related to the physical structure and adsorptive capacity of the granular carriers. When the physical structure of attapulgite granules was changed by moistening with water, vapor activity increased. Carriers that did not change in physical structure on contact with water exhibited unchanged or reduced vapor activity. Danielson proposed the use of impervious granular carriers to obtain immediate short-term activity of CIPC and more adsorptive carriers for long-term activity.

Adsorption.--The activity of most herbicides varies with soil composition. Since many herbicides are adsorbed by colloidal particles and since the amounts of mineral and organic colloids vary among soils, much of the variation in herbicidal activity is attributed to differences in the adsorptive capacity.

The adsorption of six growth-regulator herbicides by several ion-exchange resins was demonstrated by Weaver (59). Weaver (60) and Smith and Ennis (53) used activated charcoal as a soil amendment to protect germination seeds from 2,4-D applied to the soil surface.

In a greenhouse experiment the initial toxicity of 2,4-D was greater in sandy soils than in most clay soils (16). A butyl ester of 2,4-D was fixed in a clay-sand mixture more strongly than a triethanolamine salt form (2). Both amine and polypropylene ester formulations of 2,4-D were adsorbed by montmorillonite, illite, and kaolinite clays (32). The adsorption of 2,4-D increased as the cation-exchange capacity and specific surface increased.

CIPC was adsorbed by activated charcoal and certain other materials (17). In laboratory and greenhouse experiments EPTC was adsorbed least by those soils in which it was most phytotoxic (5).

Sherburne and Freed (51) demonstrated adsorption of monuron by activated charcoal, sawdust, straw, and soil. The amount of monuron adsorbed by soils was correlated with

organic matter and clay content. Studies by Hill (32) showed that the clay content, type of clay, and organic matter of soils influenced the amount of monuron adsorbed. Adsorption increased as clay content or organic matter increased. Approximately 150 p.p.m. was required on a bentonite clay to give 1 p.p.m. in the soil solution whereas less than 1 p.p.m. was required on a kaolinitic clay to give 1 p.p.m. in solution.

The herbicidal activity of the phenylureas was correlated inversely with soil organic matter, total clay, and cation-exchange capacity (47, 56). Multiple regression analyses suggested that soil organic matter was most important in toxicity reduction of monuron, diuron, 3-phenyl-1,1-dimethylurea [fenuron], and 3-(3,4-dichlorophenyl)-1-methylurea [DMU].

Variations in the effective dosage ranges among several soils suggested greatest adsorption of diuron and DMU and least adsorption of monuron and fenuron (47). Coggins and Crafts (15) showed that clay suspended in solutions of the phenylurea herbicides reduced the toxicity to barley. The toxicity of 1-n-butyl-3-(3,4-dichlorophenyl)-1-methylurea [neburon] was altered most, and alteration of toxicities of DMU, diuron, monuron, and fenuron followed. In general, water solubility and adsorption were inversely related. In a recent report Leopold, et al. (37) demonstrated an inverse relation between solubility of several chlorinated phenoxyacetic acids and their adsorption on charcoal.

In an aqueous medium 2-chloro-4,6-bis(ethylamino)-s-triazine [simazine] was adsorbed to a cation exchanger and to activated charcoal but not to an anion exchanger (48). Soil toxicity tests with the s-triazines suggested considerable variation in soil adsorption of these compounds. The effect of soil organic matter, clay content, cation-exchange capacity, and pH on the phytotoxicity of simazine was investigated in detail. Soil organic matter appeared to alter the initial toxicity of simazine in soils most.

In soil systems adsorbed herbicides are probably gradually desorbed as leaching, chemical, and biological degradation; and absorption by plants reduce the concentration in the soil solution. However, the adsorption-desorption relations of herbicide molecules in soils and the importance of these phenomena in the movement of herbicides in soils have not been adequately investigated.

Leaching.--The movement of herbicides in soils depends on or is influenced by several factors. Upchurch and Pierce (57, 58) indicated that at least two steps are involved in the movement of an herbicide downward in soil: (a) Entrance of the herbicide into solution and (b) adsorption of the herbicide to soil particles. Entrance into solution could occur from solid particles of the herbicide or from colloidal particles with adsorbed herbicide molecules. These two processes, solution and adsorption, may be affected by several variables.

The solubilities of herbicides and of salts of herbicides that may form in the soil are important properties affecting leaching (32, 41, 43). Minarik (41) discussed the leachability of 2,4-D and its salts. He pointed out that the calcium, magnesium, potassium, sodium, and ammonium salts of 2,4-D are more soluble in water than the acid, whereas salts of heavy metals such as iron and copper are less soluble than the acid. The equilibrium status of the several forms of 2,4-D in the soil probably affects the leaching rate of 2,4-D. However, Smith and Ennis (53) did not measure a difference in the movement of the acid, the triethanolamine salt, and the sodium salt of 2,4-D in soils. Hill (32) concluded that the lower water solubility of diuron compared to monuron resulted in slower movement of diuron that monuron in soils. Diuron is adsorbed more strongly than monuron and differences in adsorption probably contribute to differential leaching of these two compounds.

An herbicide that is strongly fixed in soils should leach less readily than one that is not so tenaciously fixed (2). The adsorptive capacity of soil is influenced by soil organic matter and the amounts and types of clay minerals. The adsorption process is influenced

by temperature and the nature of the solvent. The adsorptive characteristics of a compound are influenced by pH of the solution. Since these factors influence adsorption, they must influence movement of herbicides in soils. The organic matter content and soil texture are known to influence leaching (20, 24, 25, 26, 32, 35, 44, 47, 52, 53, 58).

The leachability of DNBP appeared to be influenced by pH. Dowler, et al. (24) concluded that movement of DNBP in soils was as much a function of soil type and soil reaction as of the amount of rainfall. In experiments by Upchurch and Pierce (58) soil temperatures of 5° to 45° C. had little effect on the monuron leached from the upper 2-inch layer of soil columns. However, greater amounts of monuron were retained by the 2- to 8-inch layer at 25°, 35°, and 45° C. than at 5° and 15° C.

That the amount of rainfall or of water applied as irrigation influences the movement of herbicides has been demonstrated by many research workers. Sherburne, et al. (52) compared the movement of monuron in soil columns to the movement of compounds in chromatography and concluded that the depth of the highest concentration of the herbicide in soil columns was a function of the amount of water added to the soil surface. Upchurch and Pierce (57) studied the effect of amount, intensity, and frequency of simulated rainfall on the leaching of monuron. The greater the amount of simulated rain the greater the movement of monuron. Rainfall intensities of 1/16 to 4 inches per application had little influence on the amount of monuron retained in the top 2-inch layer. In the 2- to 8-inch zone greater accumulation of monuron occurred with low intensities than with high. A greater movement of monuron from the upper soil layers occurred as frequency of rainfall increased. Approximately half of the frequency effect was attributed to evaporation of water from the surface of soil columns that received less frequent applications. These workers concluded that of the three variables studied, the amount of rainfall would be most directly correlated with the distribution of herbicides in soil profiles under field conditions although they maintained that intensity and frequency might also be of practical importance.

The effect of the amount of monuron applied on the amount moved by simulated rainfall was also investigated by Upchurch and Pierce (58). Monuron was applied to surfaces of soil columns at rates of 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 lb./A, and the applications were followed by 4 inches of simulated rainfall. The lowest percent retention (34 percent) in the 0- to 2-inch layer was found in columns treated with 32 lb./A. Retention by the 2-inch layer was increasingly greater as the rate increased and decreased from 32 lb./A. These workers suggested that the increasing percent retention when the rate of application was increased from 32 to 256 lb./A was attributable to the low solubility of monuron.

Molecular size may also be a factor in the movement of herbicides in soils (15, 44).

The exact pattern of movement of an herbicide in a particular soil would be impossible to predict presently. However, relative rates of movement can be predicted for many herbicides.

Chemical reaction.--The formation of salts of 2,4-D and DNBP and a possible reaction scheme for inactivation of 2,4-D in soils have been discussed. Relatively little is known of the chemical reactions that most herbicides undergo in soils. Hydrolysis, oxidation, and formation of complexes are known reactions for certain herbicides.

Amitrole forms stable complexes with cobalt, copper, nickel, iron, and magnesium. Sund (54) suggested that complexing with metal ions in the soil solution was one mechanism by which amitrole was detoxified.

The 2-chloroacetamides, for example 2-chloro-N,N-diallylacetamide [CDAA], can be hydrolyzed in the soil (31). The chlorine atom and the amide linkage are sites on the molecule where hydrolysis may occur. Regardless of the site where hydrolysis begins, the end products are glycolic acid and secondary amines.

The dithiocarbamates, for example 2-chloroallyl diethyldithiocarbamate [CDEC], can be broken down in the soil by oxidation and hydrolysis (31). If hydrolysis of CDEC precedes oxidation, allyl alcohol is an intermediate; whereas if oxidation precedes hydrolysis, 2-(diethyldithiocarbamyl)acetic acid is an intermediate. The two reactions can occur separately or concurrently in the soil. End products of breakdown are formic acid, glycolic acid, carbon disulfide, and secondary amines for both reaction pathways. The breakdown products may undergo further reaction in the soil.

Freed, et al. (29) suggested that EPTC was hydrolyzed in water; and according to the reaction scheme which they proposed, a secondary amine, carbon dioxide, and ethylmercaptan were end products.

Soil treated with 3,5-dimethyltetrahydro-1,3,5,2 H-thiadiazine-2-thione [DMTT] evolves formaldehyde, which is thought to be the first product of DMTT breakdown in the soil (40). Methylaminomethyldithiocarbamate forms next and activation continues by forming monoethylamine, methyl isothiocyanate, and hydrogen sulfide. Monoethylamine and hydrogen sulfide react with formaldehyde and form methylaminoethanol, dimethylaminomethane, and 1,3,5-trithiocyclohexane. Eventually this reaction proceeds to carbon dioxide, ammonia, sulfur dioxide, and water. The methyl isothiocyanate and water react to give carbon dioxide, bydrogen sulfide, and methylamine; and the methylamine degrades into carbon dioxide and ammonia.

Monuron is thought to be hydrolyzed slowly in the soil to p-chloroamiline (45).

In the presence of moisture tris-(2,4-dichlorophenoxyethyl)phosphite [2,4-DEP] is slowly hydrolyzed step-wise to form one mole of phosphorous acid and three moles of 2,4-dichlorophenoxyethanol (28).

The reactions which herbicides and agricultural pesticides in general undergo in soils and the products formed are important with respect to residues in soils. Weed research scientists should emphasize this phase of herbicide research.

Photodecomposition.--Less is known about the direct effect of light on the breakdown of herbicides than other factors suspected of being involved. However, photodecomposition of monuron was demonstrated by Hill, et al. (33). When a solution containing 88.3 p.p.m. of monuron sealed in quartz tubes was exposed for 48 days to sunlight, an 83-percent loss of monuron occurred. Hill, et al. (33) concluded that in dry areas of the Western United States monuron may be inactivated by ultraviolet irradiation. They suggested that this factor would account for disappearance of only a small part of the herbicide in humid regions where frequent rains move it into soils.

Neburon, diuron, monuron, fenuron, and DMU were applied as alcohol solutions to filter paper (14). After the paper dried, it was exposed to ultraviolet light for several hours. The herbicides were not visible prior to exposure, but in white light they were readily visible after exposure as light tan spots. The compounds were apparently changed during exposure.

The effects of shade, moisture, and position in the soil on the residual activity of monuron, diuron, and simazine were investigated in cooperation with the California Agricultural Experiment Station. The activity of monuron and diuron disappeared more rapidly from soil exposed to the sun than from shaded soil. The activity of monuron and simazine disappeared more rapidly from moist soil than from dry soil. Monuron, diuron, and simazine were not affected to the same degree by these variables. Soil temperature was measured but not controlled in this experiment, and soil temperatures varied considerably among the treatments during the day. Temperature markedly influences vapor pressure and chemical reactions. Therefore the difference in the rate of disappearance in shaded soil and soil exposed to the sun cannot be attributed unquestionably to light inactivation.

Absorption by plants.--Herbicides are absorbed by plant roots and are usually translocated to the aerial parts. Within the plant the herbicide molecules are subjected to various physical and chemical processes. Crop plants may be removed from the land or they may be returned to the soil along with weed growth. Most plant roots remain in the soil. Therefore a portion of herbicides absorbed by plants and the metabolic products of herbicides in plant tissues may eventually be returned to the soil.

It has been stressed previously that soils reduce the initial effectiveness of herbicides and that the degree of effect varied among soil types and herbicides. Unpublished data showed that at least four times as much simazine was required in a clay loam soil as in solution culture to produce the same weight reductions of seedling oats. In another experiment the dry-weight increase of oat tops following simazine treatment through the roots was reduced 50 percent by 7.2 mg. of the herbicide (C^{14} expressed as simazine) per gram of dry tissue at harvest 9 days after initial exposure (49). The amount of simazine (C^{14} expressed as simazine) required in seedling oat plants to reduce plant weight was less than 2 percent of that present in 400 ml. of the 0.05 p.p.m. by weight culture solution initially. Although the conditions of this experiment were markedly different from those which occur in the field, plants probably absorb only a small fraction of the total amount of an herbicide applied to the soil.

CONCLUSIONS

Although considerable progress has been made, much additional information is needed on the fate of herbicides in soils. Weed research scientists need to know more about the persistence of herbicides in soils under varying environmental conditions so that they can establish safe rotational practices. Mammalism toxicity of some soil degradation products should be determined, because these products can also be absorbed by plants. Information on adsorption-desorption relationships; on the interrelationships of adsorption, volatility, solubility, and leaching of herbicides; on the nature and extent of microbial and chemical inactivation; on the importance of photodecomposition; and on the influence of various environmental factors on these processes is essential to an understanding of the behavior of herbicides in soils.

Weed scientists should determine the component or components of the soil from which dosage requirements of soil-applied herbicides can be predicted. Some of this information is available (48, 56), but more is necessary. Eventually specific recommendations of rates, times, and methods of application of herbicides may be based on weather forecasts and analyses of soil samples from farmers' fields (25).

One of the most urgent needs for research on the fate of herbicides in soils is methods of isolation and identification of herbicides and breakdown products. Biological and chemical assays must be improved and new ones devised. Radioactive isotopes have been used very little to study the decomposition of soil-applied herbicides. Soil samples could be treated with different lots of an herbicide with each lot tagged with C^{14} at different positions in the molecule. By known analytical techniques the unchanged herbicide and many reaction products could be separated and identified. Radioisotopes should be most useful tools in future research of this nature.

As new herbicides are developed, their behavior in soils in response to variable soil characteristics, weather conditions, and cultural practices must be investigated concurrently with some of the more fundamental aspects prior to recommendations for use by farmers.

LITERATURE CITED

1. Aldrich, R. J. Herbicides, residues in soils. Agric. and Food Chem. 1: 257-260. 1953.

2. _____ and Willard, C. J. Factors affecting the pre-emergence use of 2,4-D in corn. Weeds 1: 338-345. 1952.

3. Alexander, M. and Aleem, M.I.H. Effect of chemical structure on microbial decomposition of aromatic herbicides. (Manuscript submitted to Agric. and Food Chem.) 1960.

4. Anderson, W. P., Linder, P. J., and Mitchell, J. W. Evaporation of some plant growth regulators and its possible effect on their activity. Science 116: 502-503. 1952.

5. Ashton, F. M. and Sheets, T. J. The relationship of soil adsorption of EPTC to oats injury in various soil types. Weeds 7: 88-90. 1959.

6. Audus, L. J. Biological detoxication of 2,4-dichlorophenoxyacetic acid in soils: isolation of an effective organism. Nature 166: 356. 1950.

7. _____ . The biological detoxication of hormone herbicides in soil. Plant and Soil 3: 170-192. 1951.

8. _____ . Plant Growth Substances. Leonard Hill Limited, London. 1959.

9. Barrons, K. C., Lynn, G. E., and Eastman, J. D. Experiments on the reduction of high temperature injury to cotton from DNOSBP. Proc. SWC 6: 33-37. 1953.

10. Bleidner, W. C., Baker, H. M., Levitsky, M., and Lowen, W. K. Determination of 3-(p-chlorophenyl)-1,1-dimethylurea in soils and plant tissue. Agric. and Food Chem. 2: 476-479. 1954.

11. Burschel, P. and Freed, V. H. The decomposition of herbicides in soils. Weeds 7: 157-161. 1959.

12. Carroll, R. B. Factors influencing the activation of 2,4-dichlorophenoxy-ethyl sulfate. Proc. SWC 4: 13. 1951.

13. _____ . Activation of sodium 2-(2,4-dichlorophenoxy)ethyl sulfate. Contrib. Boyce Thomp. Inst. 16: 409-417. 1952.

14. Coggins, C. W. Department of Horticulture, University of California, Riverside, California, personal communication. Nov. 6, 1957.

15. _____ and Crafts, A. S. Substituted urea herbicides: Their electrophoretic behavior and the influence of clay colloid in nutrient solution on their phytotoxicity. Weeds 7: 349-358. 1959.

16. Crafts, A. S. Toxicity of 2,4-D in California soils. Hilgardia 19: 141-158. 1949.

17. Danielson, L. L. Mode and rate of release of isopropyl N-(3-chlorophenyl)-carbamate from several granular carriers. Weeds 7: 418-426. 1959.

18. _____ , Gentner, W. A., and Jansen, L. L. Research in progress, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland. 1960.

19. Davis, D. E. Some factors that affect the phytotoxicity of water soluble DNBP. Weeds 4: 227-234. 1956.

20. Davis, F. L. and Selman, F. L. Effects of water upon the movement of dinitro weed killers in soils. Weeds 3: 11-20. 1954.

21. DeRose, H. R. Persistence of some plant growth regulators when applied to the soil in herbicidal treatments. Bot. Gaz. 107: 583-589. 1946.

22. _____ . Crabgrass inhibition with O-isopropyl N-(3-chlorophenyl)-carbamate. Agron. Journ. 43: 139-143. 1951.

23. _____ and Newman, A. S. The comparison of the persistence of certain plant growth regulators when applied to soil. Soil Sci. Soc. Am. Proc. 12: 222-226. 1948.

24. Dowler, C., Baughman, N. M., and Veatch, C. The effect of soil type, soil pH, and simulated rainfall on the distribution of DNBP in the soil. Weeds 6: 281-288. 1958.

25. Ennis, W. B., Jr. Some soil and weather factors influencing usage of preemergence herbicides. Proc. Soil Sci. Soc. of Florida 14: 130-139. 1954.

26. _____ . Weed control in principal crops of the southern United States. Adv. in Agron. 7: 251-297. 1955.

27. Evans, W. C., and Smith, B. S. W. The photochemical inactivation and microbial metabolism of the chlorophenoxyacetic acid herbicides. Biochem. Jour. Vol. 57. 1954.

28. Feldman, A. W., Technical summary on Falone. Naugatuck Chemical Division, United States Rubber Company, Naugatuck, Connecticut. Undated.

29. Freed, V. H., Montgomery, M., and Traegde, S. C. Physical properties of S-ethyl-di-n-propylthiolcarbamate. Res. Prog. Rpt., WWCC, pp. 89-90. 1958.

30. Gard, L. N., and Rudd, N. G. Herbicides determination: isopropyl N-(3-chlorophenyl) carbamate (CIPC) in soil and crops. Agric. and Food Chem. 1: 630-632. 1953.

31. Hannah, L. H., Field studies with two new classes of herbicidal chemicals. Proc. SWC 8: 316-321. 1955.

32. Hill, G. D., Soil factors as related to herbicides action. Paper presented before The Weed Society of America. New York. 1956.

33. _____ , McGahen, J. W., Baker, H. M., Finnerty, D. W., and Bingeman, C. W. The fate of substituted urea herbicides in agricultural soils. Agron. Journ. 47: 93-103. 1955.

34. Hollingsworth, E. B. and Ennis, W. B., Jr. Some studies on vapor action of certain dinitro compounds upon young cotton plants. Proc. SWC 6: 23-31. 1953.

35. Holstun, J. T., and Loomis, W. E. Leaching and decomposition of 2,2-dichloro-propionic acid in several Iowa soils. Weeds 4: 205-217. 1956.

36. Jensen, H. L., and Petersen, H. I. Detoxication of hormone herbicides by soil bacteria. Nature 170: 39-40. 1952.

37. Leopold, A. C., Van Schaik, P., and Neal, Mary. Molecular structure and herbicide adsorption. Weeds 8: 48-54. 1960.

38. Linder, P. J., Shaw, W. C., and Marth, P. C. A comparison of the relative vapor activity and the relative rates of evaporation of several carbamates. Proc. SWC 8: 306-308. 1955.

39. Marth, P. C. and Mitchell, J. W. Comparative volatility of various forms of 2,4-D. Bot. Gaz. 110: 632-637. 1949.

40. McKenzie, R. E. Activation of mylone, a temporary soil sterilant. Res. Prog. Rpt., WWCC, p. 105. 1957.

41. Minarik, C. E. Pre-emergence herbicides and their behavior. Proc. NEWCC (sup.) 5: 29-39. 1951.

42. Newman, A.S., and Thomas, J. R. Decomposition of 2,4-dichlorophenoxyacetic acid in soil and liquid media. Soil Sci. Soc. Am. Proc. 14: 160-164. 1949.

43. Norman, A. G., and Newman, A. S. The persistence of herbicides in soils. Proc. NEWCC 4: 7-12. 1950.

44. Ogle, R. E., and Warren, G. F. Fate and activity of herbicides in soils. Weeds 3: 257-273. 1954.

45. Rahn, E. M., and Baynard, R. E., Jr. Persistence and penetration of monuron in asparagus soils. Weeds 6: 432-440. 1958.

46. Ready, D., and Grant, V. Q. A rapid sensitive method for determination of low concentrations of 2,4-dichlorophenoxyacetic acid in aqueous solutions. Bot. Gaz. 109: 39-44. 1947.

47. Sheets, T. J. The comparative toxicities of four phenylurea herbicides in several soil types. Weeds 6: 413-424. 1958.

48. _____ . The uptake, distribution, and phytotoxicity of 2-chloro-4,6-bis(ethylamino)-s-triazine. Doctoral thesis, Univ. of Calif., Davis, California. 1959.

49. Sheets, T. J. The toxicity of simazine to seedling oat plants (Manuscript submitted to Weeds). 1960.

50. _____, and Crafts, A. S. The phytotoxicity of four phenylurea herbicides in soil. Weeds 5: 93-101. 1957.

51. Sherburne, H.R., and Freed, V. H. Adsorption of 3(p-chlorophenyl)-1,1-dimethylurea as a function of soil constituents. Agric. and Food Chem. 2: 937-939. 1954.

52. _____ , Freed, V. H., and Fang, S. C. The use of C^{14} carbonyl labeled 3(p-chlorophenyl)-1,1-dimethylurea in a leaching study. Weeds 4: 50-54. 1956.

53. Smith, R. J., Jr., and Ennis, W. B., Jr. Studies on the downward movement of 2,4-D and 3-chloro-IPC in soils. Proc. SWC 6: 63-71. 1953.

54. Sund, K. A. Residual activity of 3-amino-1,2,4-triazole in soils. Agric. and Food Chem. 4: 57-60. 1956.

55. Swanson, C. R., Shaw, W. C., and Hughes, J. H. Some effects of isopropyl N-(3-chlorophenyl)carbamate and an alkanolamine salt on germinating cotton seeds. Weeds 2: 178-189. 1953.

56. Upchurch, R. P. The influence of soil factors on the phytotoxicity and plant selectivity of diuron. Weeds 6: 161-171. 1958.

57. _____ and Pierce, W. C. The leaching of monuron from Lakeland sand soil. Part I. The effect of amount, intensity, and frequency of simulated rainfall. Weeds 5: 321-330. 1957.

58. _____ and Pierce, W. C. The leaching of monuron from Lakeland sand soil. Part II. The effect of soil temperature, organic matter, soil moisture, and amount of herbicide. Weeds 6: 24-33. 1958.

59. Weaver, R. J. Reaction of certain plant growth regulators with ion exchangers. Bot. Gaz. 109: 72-84. 1947.

60. _____ . Some uses of activated carbon in contratoxification of plant growth regulators. Bot. Gaz. 110: 300-312. 1948.

61. Whiteside, Jean S., and Alexander, M. Measurement of microbiological effects of herbicides. Weeds 8: 204-213. 1960.

62. Young, H. C., and Carroll, J. C. The decomposition of pentachlorophenol when applied as a residual pre-emergence herbicide. Agron. Jour. 43: 504-507. 1951.

GROWTH REGULATORS AND THERAPEUTANTS...THEIR ABSORPTION, TRANSLOCATION, AND METABOLISM IN PLANTS

John W. Mitchell, Paul J. Linder, and Bernard C. Smale[1]

INTRODUCTION

The fate of both growth regulators and therapeutants depends not only on the kind of compound involved, but also on where the compound is applied to the plant. For example, when a growth regulator was applied to the upper part of a bean stem, five times as much of the chemical was found inside the plant as when the lower, more mature part of the stem was treated (40). Or in the case of therapeutants, when one of the commercially used antibiotics was applied to cherry leaves, about 2 weeks were required for the compound to be inactivated, but when applied to the fruits the therapeutant was inactivated within a few days (18, 54). It is thus clear that site of application affects to some extent the fate of regulators and therapeutants. We will consider, therefore, the fate of regulating chemicals and therapeutants in relation to the site of application as well as in relation to the kind of compound used.

GROWTH REGULATORS

Growth-regulating chemicals cannot bring about desirable effects as long as they remain on the surface of a plant. All growth regulators are absorbed and most of them are readily translocated in the phloem of plants. Translocation therefore figures prominently in the fate of these substances. Fortunately, from the standpoint of residues, only very minute amounts of regulating compounds are required to bring about useful responses in crop plants. Furthermore, regulating compounds as a class must be relatively nontoxic in order to be most effective for controlling the growth and behavior of plants.

[1] Physiologists and Pathologist, respectively, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

Applied to Leaves

As far as we know, all regulating compounds are absorbed by leaves and most of them are translocated to other parts of plants (43). Absorption and translocation are favored by such conditions as relatively high light intensity, high humidity, and moderate temperatures (10, 12, 20, 27, 33, 38, 58). It has long been known that movement of regulating chemicals from the leaves to other parts of the plant is associated with movement of photosynthate in the same direction (38, 63). The rate at which regulating compounds are absorbed and translocated from the leaves can be accelerated under certain conditions by the addition of such additives as surfactants, sugar, and boron (14, 20, 24, 36, 39, 48).

A few regulating compounds are not readily translocated out of leaves. For example, isopropyl-N-phenylcarbamate was not absorbed and translocated in effective amounts from leaves of the Wong barley variety (46). 2, 4-dichlorobenzyl nicotinium sulfate, a regulator that suppresses growth, was not readily translocated from the leaves of bean plants (49).

The fate of regulating compounds depends to some extent on the stage of maturity of the leaves to which they are applied. Experiments with radioactively tagged regulating compounds have shown that relatively immature partly expanded leaves may absorb these substances, but these young leaves were unable to translocate effective amounts of the chemicals to other parts of the plant. In a similar way, relatively old leaves do not absorb and translocate regulating compounds to other parts of the plant as effectively as do leaves in their prime (42). Metabolism of regulating substances by leaves in these different stages of maturation has not been studied.

Most regulating compounds are moved in both an upward and a downward direction after translocation to the stems. The final distribution pattern, however, depends to some extent upon the type of regulating chemical used. Some, such as 2-iodo-3-nitrobenzoic acid (or its metabolites), accumulated in the tips of bean plants after application to the leaves while 2, 4-dichloro-5-iodophenoxyacetic acid (or its metabolites) accumulated mainly in the lower stem (43).

Some regulating chemicals move from leaves and accumulate more readily in the aboveground parts of the plant than in their roots (20, 41). There is evidence that certain regulating chemicals may accumulate in the seeds of plants after application to the leaves. For example, a growth-suppressing chemical known as Amo-1618 applied to the leaves of bean plants accumulated in the seeds in sufficient amounts to suppress greatly the growth of plants that develop from these seeds. The regulator accumulated in the seeds either as the compound applied to the plant or as a metabolite capable of inducing identical growth responses (35).

The fate of growth regulators as far as their absorption and translocation is concerned depends to some extent upon the molecular structure of the compound involved. It recently has been possible to accelerate absorption and translocation of some regulating chemicals, or of their metabolites, by slightly altering their structural configurations. For example, the absorption and translocation of phenylacetic acid, 2,4-dichlorophenylmercaptoacetic acid, isopropyl-N-phenylcarbamate and naphthaleneacetic acid were enhanced by modifying the parent compounds to lactic acid derivatives (43, 44). The increase due to this change varied from about 30 percent for the mercapto compound to 600 percent for naphthaleneacetic acid.

With respect to the metabolism of regulators when applied to the leaves of plants, some of these compounds apparently enter the plant, remain, in part at least, essentially unchanged as they move from the leaves to other parts. For example, alpha-methoxyphenylacetic acid was absorbed by leaves, and then was translocated to the roots and exuded into surrounding aerated water without detectable change (30, 31, 55). Naphthaleneacetic acid applied to leaves of bean plants grown in light from fluorescent tubes was absorbed and translocated to the stems without any detectable change (44). Minute amounts of both the methoxy acid and the naphthalene acid reacted with some plant

constituents, however, since both induced growth regulation but the amounts involved in this reaction were not detectable.

Other regulating compounds are only partially metabolized when moderate amounts are placed on leaves. Holley, et al. (22) reported, for example, that approximately 30 percent of the 2,4-dichlorophenoxyacetic acid absorbed by bean plants was detected in an unchanged form. Partial metabolism of this and other regulators by several kinds of plants has also been observed (15, 20, 52).

Weintraub, et al. (64), applying very small amounts of 2,4-D to the terminal buds of bean plants, found that this compound was metabolized into other compounds within a few days. Radioactive carbon originally in the regulator was detected in many kinds of plant constituents including acids, sugars, dextrins, starch, pectin, proteins, and cell-wall substances. Various investigators have shown that indoleacetic acid is broken down by oxidase enzymes of some plants, and that indolealdehyde and perhaps other products result (57, 59).

Some regulating chemicals are changed so readily within the plant that it is difficult to recover the compound originally applied. Recently C^{14}-tagged naphthalenelactamide was used to study the fate of a compound of this type. The amide was applied quantitatively and spread evenly over the upper surface of bean leaves. Metabolism of the regulator was studied as it moved from the leaves to other parts of the plant. After 2 days, midribs of the treated leaves were assayed to determine the presence of the original compound, naphthalenelactamide, and its reaction products. Petioles of the treated leaves and stems of the plants were also studied.

Naphthalenelactamide was not detected in the main veins, petioles, or stems of plants. The main veins did contain, however, one detectable reaction product and this gave an Rf of 0.67 compared with 0.92 for the lactamide applied to the leaves. This initial reaction product was also detected in the petioles and the stems. However, two additional reaction products were also detected in the petioles and these gave widely different Rf values. The stems contained still a fourth compound that differed from the parent lactamide and all the other reaction products detected (44).

It is thus evident that some regulating compounds at least may react almost completely when applied in small amounts to leaves. The primary reaction product of naphthalenelactamide found in the leaves was further altered as it reached the petioles and stems. These results parallel those of others who found only metabolites of 2,4-D in roots while 2,4-D and its metabolites were found in stems (15, 20).

Applied to Stems

Regulating compounds appear to be more readily absorbed and translocated when applied to young parts of stems than when applied to relatively mature parts. For example, when equal amounts of I^{131}-tagged 2,4-dichloro-5-iodophenoxyacetic acid were applied to relatively mature bean stems near the soil level and to relatively immature parts of the stems several inches above the soil level, most radioactivity was detected in the root when the more distant immature part of the stem was treated (40).

Metabolism of some growth-regulating chemicals has been studied following absorption by detached segments of stems (60). Most of this research has been directed mainly toward an understanding of the reactions involved when a regulator induces cell elongation rather than an understanding of the overall fate of these compounds when applied to the aerial or underground parts of intact plants.

Applied to Roots

Regulating compounds have been used little as soil treatments except experimentally. In considering the fate of some regulating compounds applied to the soil surface, Linder (29) and Muzik (53) found that 2,4-D was absorbed relatively near the surface of the soil.

It is well known that many regulating compounds are readily degraded when in contact with moist soil (2, 56). Soil microorganisms probably account for most of this degradation. Moisture plays an important role in governing the rate at which regulators are broken down in soils. For example, 2,4-D remained biologically active for only about 2 weeks when stored in moist soil at the rate of 20 mg. per pound of soil whereas in dry soil it remained active for $1\frac{1}{2}$ years (45).

Some regulating compounds, on the other hand, remain active when mixed with moist soil for long periods. For example, the quaternary ammonium compound Amo-1618 remained active in moist soil for a period of over 10 years when incorporated at a rate of 50 and 100 pounds per acre. Even 1 pound per acre remained effective for about 5 years (34).

Once absorbed by the root, regulating compounds are translocated throughout the plant mainly upward through tracheary elements (30, 31). Little research has been directed toward gaining an understanding of the metabolism of regulating compounds by roots since their application has for the most part involved aboveground parts.

Applied to Other Plant Parts

Regulating compounds are absorbed in biologically effective amounts by some kinds of fruits, since these substances hasten growth or maturity of those such as pears, apricots, apples, and bananas. Maxie and his coworkers (36) found that C^{14}-labeled 2,4,5-trichlorophenoxyacetic acid applied to intact branches of apricot trees was present in the mesocarp of the fruit in detectable amounts within 3 days after the trees had been sprayed. Within 10 days C^{14} was detected in the embryos and integuments and the amount increased until the fruit was mature. At least some 2,4,5-trichlorophenoxyacetic acid was detected in integuments of the fruit as the intact molecule.

Some regulators are used commercially to induce tomato plants to develop fruits by applying them to the flowers at the time of pollination. The fate of regulating compounds used for this purpose is now being studied. It is evident from the methods used commercially that application of the regulator to one flower cluster does not result in translocation of effective amounts of the compound from this treated cluster to untreated ones.

Regulating chemicals are also used commercially to prevent growth of vegetative buds on stored plants. As far as published information goes, research in this field has been directed mainly toward development of effective chemicals rather than a study of the fate of the compounds that are useful.

THERAPEUTANTS

Generally speaking, both antibiotics and synthetically produced therapeutants are readily absorbed by roots, stems, leaves, and fruits. Little is known concerning absorption by flowers. Therapeutant translocation is associated with water transport. Moving upward mainly in the xylem, these compounds tend to accumulate in leaves and possibly in fruits soon after application to roots or stems (1, 3, 5, 21, 32, 50, 51, 62). Therapeutants translocated into leaves or directly absorbed by leaves are not readily retranslocated (11, 48). This translocation characteristic is true of most therapeutants used at disease-controlling concentrations. The amount absorbed by leaves can be enhanced to some extent with adjuvants (21, 23, 61). Thus metabolism of therapeutants within leaves and fruits is of particular interest.

Application to Leaves

Some antibiotics are apparently changed while still on the surface of leaves as indicated by loss of microbial activity. For example, the antibiotic pimaricin was almost

completely inactivated in 3 hours when sprayed on bean leaves (9). Surface inactivation of pimaricin was thought to be the result of either oxidation or breakdown due to ultraviolet exposure. This same antibiotic after it had been absorbed and translocated into a leaf, however, was not inactivated during this period.

Some therapeutants absorbed by the leaf, however, are degraded or changed rather slowly. For instance, a relatively large amount of the actidione applied to cherry leaves was unchanged even after 3 weeks (18). Other research resulted in the recovery and identification of this compound from wheat seedlings 5 weeks after application (61). Similarly, streptomycin was detected unchanged in tobacco leaves 5 weeks after spray application (21).

On the other hand, some therapeutants absorbed by the leaf are changed more rapidly. The antibiotic F-17 was altered to such an extent in bean leaves after 5 days that all its therapeutic activity was lost (47).

The rate of inactivation of the therapeutant also depends upon the plant species. For example, vancomycin was completely inactivated in tomato and tobacco plants within 2 days while in the case of cucumber some of the antibiotic was unaltered for at least 10 days. The amount of antibiotic used is also important since when applied in larger amounts vancomycin remained unchanged in all of these plants for a relatively long time.

Most therapeutants are not readily translocated from leaves to roots. The antibiotic vancomycin, however, appears to be an exception. This compound is translocated unchanged from treated leaves to other parts of plants including roots. In fact the concentration of vancomycin that accumulated in roots of cucumber amounted to 16 micrograms per ml. of plant juice (37).

Various hypotheses have been proposed concerning the way these therapeutants may break down in leaves and also other plant parts (17).

Application to Stems

The upward movement of unaltered streptomycin applied to stems was first studied by our group (51). The therapeutant, when translocated to leaves, had a half-life of about 7 days on the basis of a bacterial assay. This upward translocation was associated with the transpiration stream and no downward movement (phloem transport) was detected.

The rate that this antibiotic moves up stems is illustrated in work on hops. Streptomycin was absorbed and moved up the stem 3 inches within 30 minutes (32).

Application to Roots

Many antibiotics are absorbed by roots and readily translocated upward to aboveground parts (4, 5, 13, 25). Some therapeutants, however, accumulate in roots and are not readily moved upward (6, 7). For example, Crowdy, et al. (4) showed that sulphaguanadine is almost completely retained by roots.

The fate of therapeutants following root treatment also varies with the kind of plant used. This is clearly shown by the rapid upward movement of streptomycin from roots of tomato contrasted with the relatively slow upward movement following absorption of this compound by roots of broad bean. In this work more antibiotic accumulated in tops of tomato than accumulated in those of broad bean plants (4). In addition, distribution of therapeutants in the aboveground parts of plants varies with the kind of plant used. For example, Crowdy (4) and Dye (13) showed that streptomycin accumulated primarily in lower leaves of peach seedlings while this same therapeutant attained highest concentrations in the upper leaves and stems of tomato.

Only limited information on the metabolism of therapeutants in roots is available. For example, all of an actidione derivative applied to tomato roots were altered, but the therapeutic part of the molecule apparently remained intact and moved upward (16, 28). An interesting similarity in the metabolism of a group of therapeutants by animals and the roots of plants has been demonstrated by Jones and Wignall (26). In animals the detoxification of sulphonamides has been attributed to acetylation of the free amino group (19). This same chemical change occurred in roots of broad bean when these compounds were used as plant therapeutants (26).

Application to Fruits

At present there is only a very limited amount of published information on the fate of therapeutants applied to fruits. Rapid inactivation of actidione occurred when very low but therapeutically effective amounts were sprayed on cherry fruits. In cool weather this therapeutant was changed much more slowly but, even so, half of it disappeared in about 24 hours (54). The rapid breakdown in the fruits contrasts sharply with the 3 weeks required when the chemical was in leaves (18).

Placed directly on isolated spores of the Penicillium that causes citrus fruit rot, the compound GS-1 killed spores at a 1 p.p.m. concentration. In contrast, even 500 times this amount of chemical failed to control the disease when the spores were on the fruit (8). This great loss in fungitoxicity is thought to be due to an enzymatic process that occurs in the fruit rind and changes in the chemical to an inactive form.

In summary, when considered as two groups of compounds, growth-regulating chemicals and therapeutants differ widely with respect to the amount required, the ability of the plant to transport these substances, and in the final distribution of the compounds in the plant. Regulating chemicals and therapeutants are alike, however, in the fact that most plants can readily absorb and metabolize both types of compounds. To use these substances most effectively, much more research is needed to understand not only the primary reactions between these compounds and plants, but also to characterize reaction products that result from their metabolism.

LITERATURE CITED

(1) Anderson, H. W., and Nienow, I. Phytopath. 37:1. 1947.

(2) Audus, L. J. Nature 166:356. 1950.

(3) Blanchard, F. A., and Diller, V. M. Am. J. Bot. 38:111. 1951.

(4) Crowdy. S. H. Ann. Appl. Biol. 45:208. 1957.

(5) Crowdy, S. H., Gardner, D., Grove, J. F., and Pramer, D. J. Exptl. Bot. 6:371. 1955.

(6) Crowdy, S. H., and Jones, D. Rudd. J. Exptl. Bot. 7:335. 1956.

(7) Crowdy, S. H., and Jones, D. Rudd. Nature 178:1165. 1956.

(8) Dekker, J., and Ark, P. A. Proc. IX International Botanical Congress, Montreal, Canada:88. 1957.

(9) Dekker, J., and Ark, P. A. Antibiotics & Chemotherapy 9:327. 1959.

(10) DeRose, H. R. and Newman, A. S. Proc. Soil Sci. Am. 12:222. 1947.

(11) Dimond, A. E., and Horsfall, J. G. Ann. Rev. Plant Physiol. 10:257. 1959.

(12) Donoho, C. W., Jr., and Mitchell, A. E. Amer. Soc. Hort. Sci. Abstract, 56th Annual Meeting. 1959.

(13) Dye, M. H. Ann. Appl. Biol. 44:567. 1956.

(14) Edgerton, L. J., and Haeseler, C. W. Proc. Am. Soc. Hort. Sci. 74:54. 1959.

(15) Fang, S. C., and Butts, J. S. Plant Physiol. 29:56. 1954.

(16) Ford, J. H., Klomparens, W., and Hamner, C. L. Publication of Upjohn Company, Kalamazoo, Mich.

(17) Gottlieb, D. Phytopath. 47:59. 1957.

(18) Hamilton, J. M., Skzolnik, M., and Sonderheimer, E. Science 123:1175. 1956.

(19) Harris, J. S., and Klein, J. R. Proc. Soc. Expt. Biol. 38:78. 1938.

(20) Hay, J. R., and Thimann, K. V. Plant Physiol. 31:446. 1956.

(21) Hidaka, Z., and Murano, H. Annals Phytopath. Soc. of Japan 21:49. 1956.

(22) Holley, R. W., Boyle, F. P., and Hand, D. B. Arch. Biochem. 27:143. 1950.

(23) Hotson, H. H. Phytopath. 43:659. 1953.

(24) Jaworski, E. G., Fang, S. C., and Freed, V. H. Plant Physiol. 30:272. 1955.

(25) Jones, D. Rudd. Outlook on Agric. 1:111. 1956.

(26) Jones, D. Rudd, and Wignall, J. Nature 175:207. 1955.

(27) Kelly, S. Plant Physiol. 24:534. 1949.

(28) Lemin, A. J., and Magee, W. E. Plant Disease Reporter 41:447. 1957.

(29) Linder, P. J. Proc. Northeastern States Weed Control Conf., New York. 1952.

(30) Linder, P. J., Craig, J. C., Jr., Cooper, F. E., and Mitchell, J. W. J. Agr. & Food Chem. 6:356. 1958.

(31) Linder, P. J., Craig, J. C., Jr., and Walton, T. R. Plant Physiol. 32:572. 1957.

(32) Maier, C. R., and Horner, C. E. Phytopath. 47:528. 1957.

(33) Marth, P. C., Davis, F. F., and Mitchell, J. W. Botan. Gaz. 107:129. 1945.

(34) Marth, P. C., and Mitchell, J. W. Proc. Amer. Soc. Hort. Sci. (In Press) 1960.

(35) Marth, P. C., Preston, W. H., Jr., and Mitchell, J. W. Bot. Gaz. 115:200. 1953.

(36) Maxie, E. C., Bradley, M. V., Robinson, B. J., and Hewitt, A. A. Proc. Amer. Soc. Hort. Sci. 73:134. 1959.

(37) Mehta, P. P., Gottlieb, D., and Powell, D. Phytopath. 49:176. 1959.

(38) Mitchell, J. W., and Brown, J. W. Bot. Gaz. 107:393. 1946.

(39) Mitchell, J. W., Dugger, W. M., Jr., and Gauch, H. G. Science 118:354. 1953.

(40) Mitchell, J. W., and Linder, P. J. Unpublished data. 1949.

(41) Mitchell, J. W., and Linder, P. J. Science 112:54. 1950.

(42) Mitchell, J. W., and Linder, P. J. Proc. Northeastern States Weed Control Conf., New York. 1950.

(43) Mitchell, J. W., and Linder, P. J. Publ. Amer. Assoc. Advance. Sci. No. 49. 1957.

(44) Mitchell, J. W., Linder, P. J., and Smale, B. C. Unpublished data. 1959.

(45) Mitchell, J. W., and Marth, P. C. Bot. Gaz. 107:408. 1946.

(46) Mitchell, J. W., Marth, P. C., and Preston, W. H., Jr. Science 120:263. 1954.

(47) Mitchell, J. W., Smale, B. C., Daly, E. J., Preston, W. H., Jr., Pridham, T. G., and Sharpe, E. S. Plant Disease Reporter 43:431. 1959.

(48) Mitchell, J. W., Smale, B. C., and Metcalf, R. L. Advances in Pest Control Research 3:359. 1960.

(49) Mitchell, J. W., Wirwille, J. W., and Weil, L. Science 110:252. 1949.

(50) Mitchell, J. W., Zaumeyer, W. J., and Anderson, W. P. Science 115:114. 1952.

(51) Mitchell, J. W., Zaumeyer, W. J., and Preston, W. H., Jr. Phytopath. 44:25. 1954.

(52) Munakata, K., and Nakai, A. J. Agr. & Food Chem. 7:176. 1959.

(53) Muzik, T. J., Loustalot, A. J., and Cruzado, H. J. J. Amer. Soc. Agron. 43:149. 1951.

(54) Prescott, G. C., Emerson, H., and Ford, J. H. J. Agr. & Food Chem. 4:343. 1956.

(55) Preston, W. H., Jr., Mitchell, J. W., and Reeve, W. Science 111:437. 1954.

(56) Quastel, J. H. Advances in Chemistry Series, No. 1, Amer. Chem. Soc.:244. 1950.

(57) Racusen, D. Arch. Biochem. and Biophys. 58:508. 1955.

(58) Rice, E. L. Bot. Gaz. 109:301. 1948.

(59) Stutz, R. E. Quart. Rept. Argonne Natl. Lab. July-August 1956.

(60) Van Overbeek, J. Bot. Rev. 25:269. 1959.

(61) Wallen, V. R., and Millar, R. L. Phytopath. 47:291. 1957.

(62) Wallen, V. R., and Skolko, A. J. Can. J. Research, C, 28:632. 1950.

(63) Weaver, R. J., and DeRose, R. H. Bot. Gaz. 107:509. 1946.

(64) Weintraub, R. L., Yeatman, J. N., Lockhart, J. A., Reinhart, J. H., and Fields, M. Arch. Biochem. and Biophys. 40:2. 1952.

RESIDUES IN MILK FROM ANTIBIOTICS USED IN MASTITIS TREATMENT

Richard W. Brown[1]

INTRODUCTION

Mastitis is one of the most costly diseases of dairy cattle. During the last 30 years, many therapeutic agents have been used to treat mastitis. However, it was not until penicillin and other antibiotics became available that a new era of treatment was started, which has grown into big business on the premise that the use of antibiotics will cure the mastitis problem. It is estimated that 75 tons of antibiotics are used yearly for the treatment of mastitis in the United States (80). Today, about 35 companies prepare products for intramammary treatment. These products are distributed or sold under about 1,500 different labels. One pharmaceutical company estimated in 1957 that the dairy industry spends $15 to $20 million a year for these preparations and that at least 80 percent of this business is in the form of over-the-counter sales to farmers. Undoubtedly, antibiotics are valuable in treating mastitis when properly used; however, there is no indication that the incidence of this disease has been decreased through their promiscuous sale and use. Since the advent of these "wonder drugs," the mastitis problem has not been solved, although their use has "cured" most interest in, support of, and ideas on fundamental mastitis research.

The problems of antibiotic contamination of milk supplies have been adequately reviewed (12, 18, 20, 52). More than 10 years ago, antibiotics in milk became a problem to the cheese manufacturing industry (23, 32, 41). To overcome the effects of the antibiotics, and particularly penicillin, increased inoculums of starter cultures were used, strains of bacteria resistant to increased concentrations of penicillin were developed, and penicillinase, an enzyme which destroys penicillin, was added to the milk (52). The cause for antibiotics in milk, that is, the widespread treatment of mastitis, was disregarded. Later, penicillin in milk was considered a potential public health problem because it might cause reactions in highly sensitive individuals (79). Only recently has it been shown that penicillin in dairy products can cause a chronic urticaria or dermatitis in sensitized persons (76, 83).

According to surveys made by the Food and Drug Administration (F.D.A.), Department of Health, Education and Welfare, 11.6 percent of the milk samples tested in 1955 contained residual penicillin; in 1956, this percentage was 5.9 (79, 80). Accordingly, certain steps taken by the F.D.A. in 1957 to alleviate the problem were: (1) The label on any antibiotic preparation intended for intramammary infusion was required to contain a statement that the milk from dairy animals must not be used for human consumption within 72 hours after the last treatment for mastitis; (2) the amount of penicillin in any mastitis preparation used for intramammary infusion was limited to 100,000 units; (3) a cooperative program with the Department of Agriculture was started to educate the farmers of the importance of the problem (35).

As a result of these measures, some progress was made in reducing the incidence of penicillin in market milk to 3.7 percent of samples tested in 1958 (35). Nevertheless, because of the failure to eliminate penicillin from market milk, the F.D.A. in 1959 enforced an existing law that permits no detectable quantity of penicillin in interstate shipments of market milk.

The amount of penicillin found in the milk samples tested during these surveys (35, 79, 80) ranged from 0.003 u./ml. to 1.2 u./ml., which would correspond to approximately 3 to 1,000 units per quart of milk or less than 1 to 275 units per glass. These

[1]Principal Research Veteranarian, Mastitis Research, National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture.

amounts are insignificant when we consider the amounts used to treat persons for various diseases. However, it apparently takes very little penicillin to cause a reaction in sensitive persons (6, 11). For instance, Bierlein (6) described a woman who developed an anaphylactic reaction when injected intradermally with 0.000003 unit of penicillin. This is at least 1,000 times more sensitive than the present microbiological test for this antibiotic. It is not too difficult to imagine that such a person would react to very low oral doses of penicillin.

Although penicillin in milk is of primary concern because it is allergenic, other antibiotics have been found in the market milk supplies and must also be considered adulterants (35, 80). Therefore, all interests may be best served by reviewing the literature and presenting information in graphic form on the persistence of the various antibiotics and sulfonamides in milk after intramammary, parenteral, and oral administration.

INTERPRETATION OF DATA

Only the maximal times of persistence and the maximal concentrations of the various antibiotics in milk are presented in the graphs. For instance, if an antibiotic in an ointment base persisted in the milk of one cow for 1 day and in another cow for 3 days, the product was recorded as having a 3-day maximal time of persistence. A number of factors influence the concentration and persistence of antibiotics in milk. Some of these factors, which are necessary for a complete interpretation of the data and could not be presented in the graphs, follow:

(1) The persistence of antibiotics differed in milk from cows in early-, mid- and late lactation (33). Whenever this information was given, only the mid-lactation data were used.

(2) The correlation was negative between milk yield and penicillin concentration (50, 75). Milk production records were not always given; therefore, they were disregarded when compiling data for the graphs.

(3) Foremilk, mid-milk and strippings contain different concentrations of antibiotics (15, 40, 58, 73). Some of the variation in antibiotic concentrations observed by various investigators, therefore, might be due to the portion of the milk assayed.

(4) Some of the investigators tested for antibiotics at lower concentrations than others; consequently, greater persistence was due, in some instances, to a greater sensitivity of the test rather than to any difference in the products.

(5) The difference in sensitivity of tests for similar antibiotics also exists. For instance, the test for Aureomycin is at least four times more sensitive than that for Terramycin; thus, some of the greater persistence of Aureomycin may be attributed to a more sensitive test.

Numerous references contained information on antibiotics and sulfonamides in milk, but they were not used in the graphs because testing for these compounds was done for limited periods of time or the doses were different from those shown in the graphs (4, 8, 39, 42, 54, 56, 64, 72, 74, 77, 78). We suggest, therefore, that the original references be consulted for complete details about these various factors.

THE INTRAMAMMARY INFUSION OF ANTIBIOTICS

Penicillin--Figure 1 shows the range of maximal times for the persistence of penicillin in milk after one intramammary infusion of 100,000 units. The data indicate that the kind of vehicle in which penicillin is suspended has an influence on its persistence in milk, although the various salts of penicillin can also influence the persistency and

may account for some of the differences shown. The oil vehicles and those with oil bases (water-in-oil emulsions and ointments) have the greatest ability to retain penicillin in the udder. The direct relationship between the amount of penicillin used in intramammary infusion and the length of time it persists in the milk is also shown in figure 1.

Mineral or peanut oil, ointments, and water-in-oil emulsions are used almost exclusively as vehicles for penicillin and other antibiotics. Consequently, the maximal times that penicillin persisted in milk when suspended in these vehicles are shown for 30 products in figure 2. Sixteen of the products caused persistence of penicillin in milk for no longer than 3 days, whereas penicillin persisted longer than 3 days when 14 of the products were used.

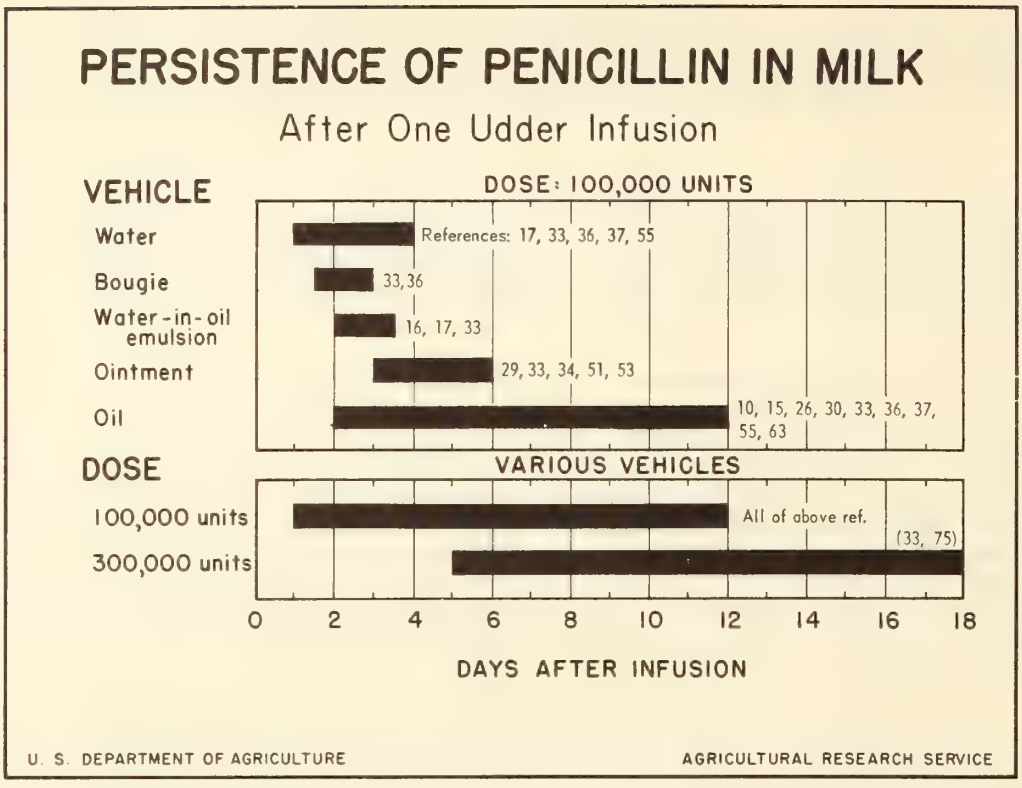
Figure 3 shows the concentration of penicillin in milk 84 to 96 hours after intramammary infusion of these latter products. From 0.03 to 0.43 units of penicillin/ml. of milk were present in the first or second milking on the fourth day after infusion. Only three of the products shown in figure 3 were produced in this country while the others were produced in Europe. In addition, three of the products contained highly insoluble salts of penicillin (benzathine and N-benzyl-2 phenylethylamine), which are not used in this country for intramammary treatment. Nevertheless, sodium, potassium, and procaine salts of penicillin are used in this country as well as vehicles with mineral oil, peanut oil, and aluminum monostearate. Recent information on oil-base products made in this country show that levels of 0.05 to 0.10 u./ml. of milk were found 96 hours after infusion and traces as long as 132 hours (5-1/2 days) (1).

One other factor, which must be considered when discarding milk from treated quarters, is that penicillin can also be excreted in the nontreated quarters. Ten cows demonstrated antibiotic residue in uninfused quarters for periods of 24 to 36 hours (1). After infusing one or more quarters, the highest concentration in any uninfused quarter was 0.05 u./ml. of milk. Negative results in untreated quarters were obtained, however, by Randall, et al (53) when one quarter was infused with 100,000 units of penicillin. Finally, there is no indication that repeated infusion of penicillin in the same quarter causes longer persistence in milk, although the data are very limited and more research on this problem is needed (37, 66, 68).

Antibiotics other than penicillin--Figure 4 shows a comparison of the persistency of some of the principal antibiotics, other than penicillin, that are used for intramammary treatment of mastitis. Although the data are limited for some of the antibiotics, there is sufficient evidence to indicate that Aureomycin has the greatest tendency to persist in milk. In fact, it might be assumed that all of the doses in ointment lasted longer than 3 days. In the four instances where it lasts only 3 days (5, 9, 47, 57) (represented by two bars in the graph), testing was stopped at that time while the levels of antibiotic were still at the relatively high concentration of 1 to 6.2 μg./ml. of milk. Terramycin and dihydrostreptomycin were also found in some instances to last 4 days. As in the case of penicillin, Aureomycin was found in the milk of untreated quarters after infusion of the treated quarters for 5 days (53), Terramycin for 1 day (53), and Neomycin for 4 hours (14).

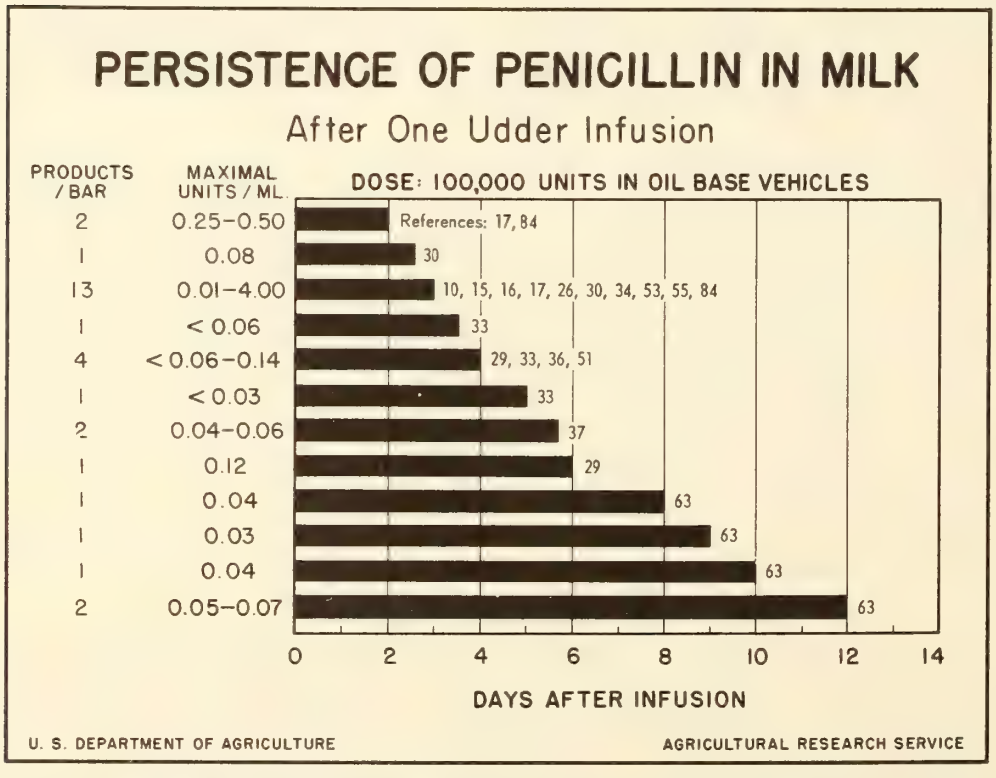
THE PARENTERAL INJECTION OF ANTIBIOTICS AND SULFONAMIDES

The treatment of mastitis with parenteral injections of antibiotics and sulfonamides is usually limited to acute cases. Contamination of market milk from such infections is no problem because the milk is usually discarded. However, dairy cattle are frequently injected with antibiotics and sulfonamides for the treatment of other diseases, and the milk is apparently normal and is used. Consequently, the farmer can inadvertently contaminate his milk supply by adding milk from these treated cows. To understand the problem better, we have summarized in figures 5, 6, and 7 the data on the persistence of various antibiotics and sulfonamides in milk after parenteral injection.



BN-10709-X

Figure 1.--Udder infusion: The effect of vehicle and dose on the persistence of penicillin in milk.


PERSISTENCE OF PENICILLIN IN MILK
After One Udder Infusion
PRODUCTS / BAR
MAXIMAL UNITS / ML.
DOSE: 100,000 UNITS IN OIL BASE VEHICLES
2 0.25–0.50 References: 17, 84
1 0.08 30
13 0.01–4.00 10, 15, 16, 17, 26, 30, 34, 53, 55, 84
1 < 0.06 33
4 < 0.06–0.14 29, 33, 36, 51
1 < 0.03 33
2 0.04–0.06 37
1 0.12 29
1 0.04 63
1 0.03 63
1 0.04 63
2 0.05–0.07 63
0 2 4 6 8 10 12 14
DAYS AFTER INFUSION
U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

BN-10708-X

Figure 2.--Udder infusion: The maximal time penicillin persisted in milk when various oil base vehicles (water-in-oil emulsion, ointment, mineral or peanut oil) were used. The maximal units per ml. refer to the concentration when last detected in milk.

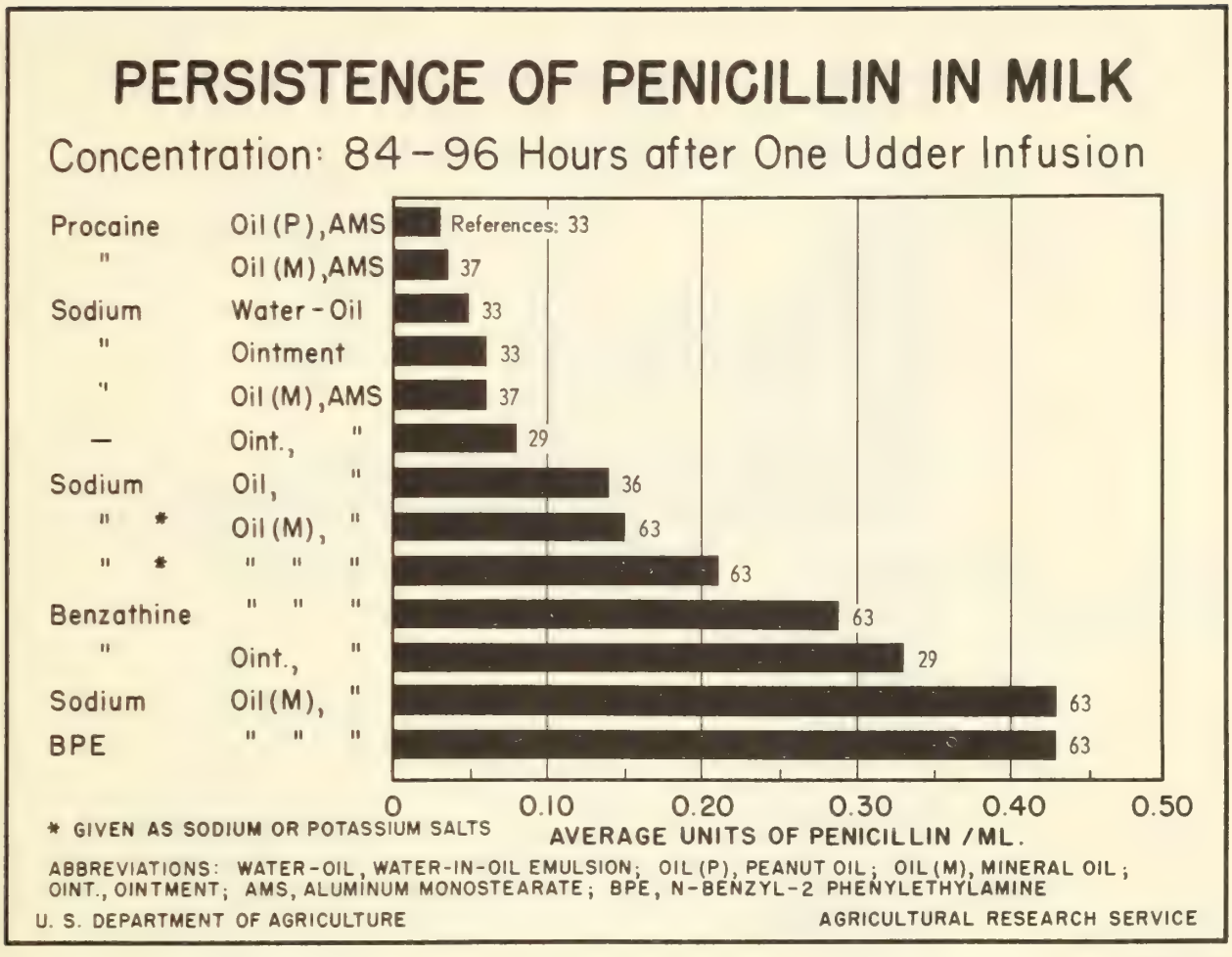


Figure 3.--Udder infusion: The average concentration of penicillin in milk when oil base vehicles were used and the residue lasted longer than 72 hours.

Penicillin--The persistence of penicillin in milk when various salts, vehicles, and doses were used is illustrated in figure 5. The penicillin products can be classified in the order of increasing persistence in milk as follows: (1) Sodium or potassium penicillin G in aqueous suspensions, (2) diethylaminoethylester penicillin G hydriodide, (3) procaine penicillin G in aqeuous suspenion, (4) procaine penicillin G in oil, and (5) benzathine penicillin G and V in aqueous suspension. The levels of penicillin in the milk several days after injection of the long persisting products were relatively low. For instance, 96 hours after injection of the procaine penicillin G in oil and the benzathine penicillin G, the average concentrations were 0.02 u./ml. of milk (82).

A direct relationship between the dose and the persistence of penicillin is also shown, although it is very slight for the first two products. A comparison of the procaine penicillin G in aqueous suspension and in oil demonstrates the marked effect oil has on the persistence of penicillin. In most instances, three successive injections at 24-hour intervals did not cause an increase in the time penicillin persisted after the last injection (28,82). None of the animals showed maximal times of persistence that exceeded those shown in figure 5. Although penicillin was not found in the milk of cows given intramuscular or intravenous injections of 1 million units or less (2,61,77), recent work (82) has shown that penicillin also did not appear in the milk of some cows injected intramuscularly with benzathine penicillin G at doses of 2,000 u./lb. of body weight and with procaine penicillin G in oil at doses as high as 5,000 u./lb. of body weight.

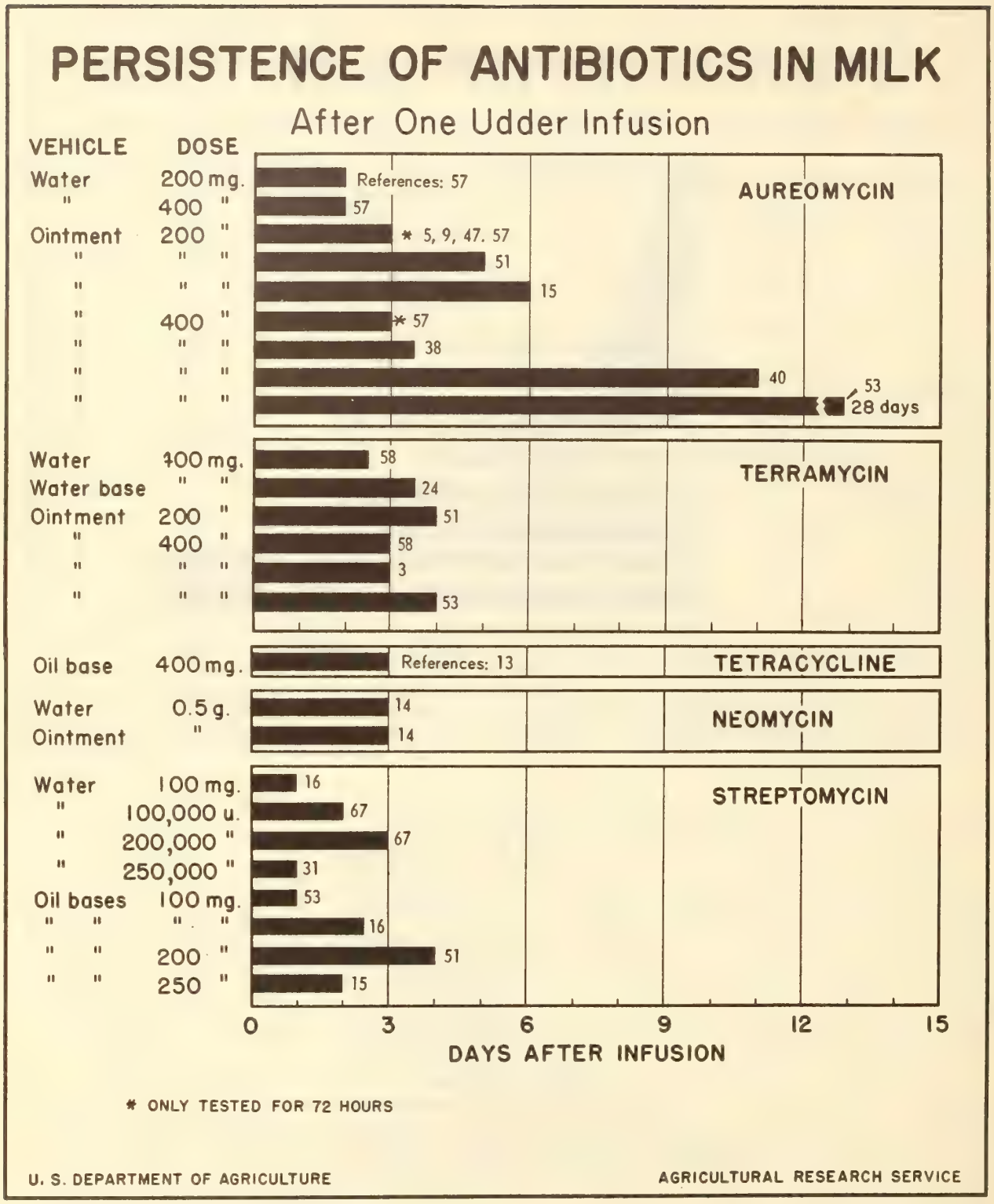
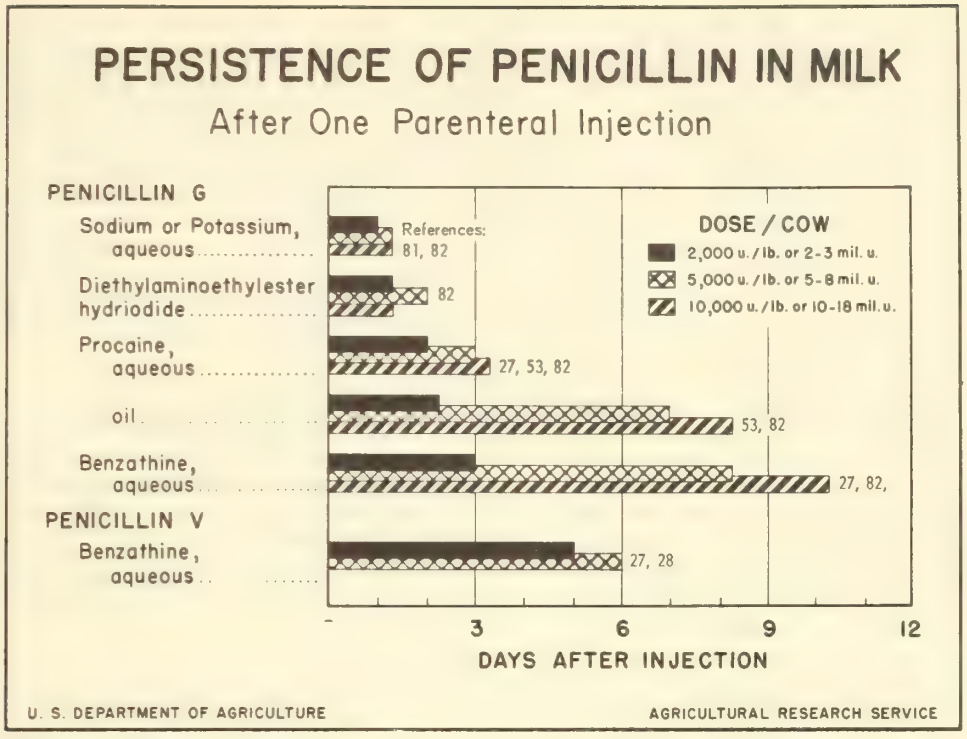
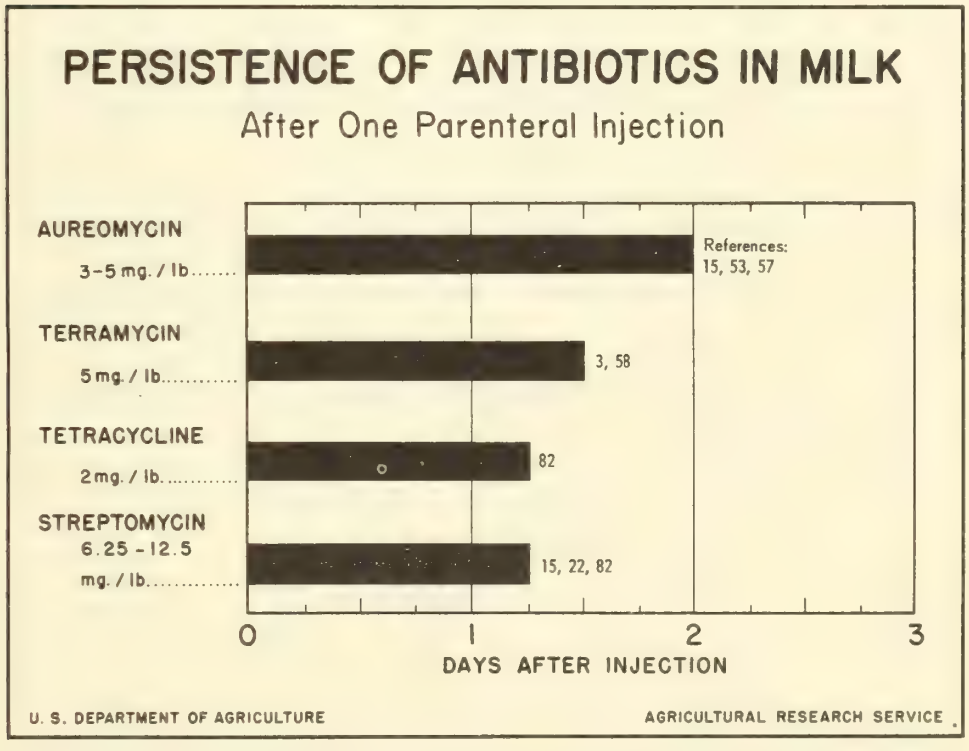


Figure 4.--Udder infusion: The maximal time various antibiotics persisted in milk when different vehicles and doses were used (Streptomycin also refers to dihydrostreptomycin.)



BN-10706-X

Figure 5.--Parenteral injection: The maximal time penicillin persisted in milk when different penicillin salts, vehicles, and doses were used.



BN-10703-X

Figure 6.--Parenteral injection: The maximal time various antibiotics persisted in milk. (Streptomycin also refers to dihydrostreptomycin.)

Antibiotics other than penicillin--Figure 6 shows a comparison of some of the other antibiotics most commonly used to treat dairy cattle diseases. Aureomycin persisted the longest in milk (2 days) after one parenteral injection, although none of the antibiotics showed any tendency to persist much longer than the shortest persisting penicillin product. Terramycin was not found in the milk of some cows given an intravenous injection of 1 gm. or intramuscular injections of 1 to 3 gm. (3,58). Tetracycline may persist longer with larger doses than the 2 mg./lb. of body weight; however, in the case of dihydrostreptomycin increasing the dose from 6.25 mg. to 12.5 mg./lb. of body weight did not increase the time of persistence (82). Three daily intramuscular injections of 6.25 mg./lb. of body weight of dihydrostreptomycin did increase the maximal time this antibiotic was found in the milk to 2-1/4 days (54 hours) (82). On the other hand, the intravenous injection of 10 gm. of Aureomycin daily for 6 days did not appear to cause any increase in the persistence of this antibiotic in milk (43).

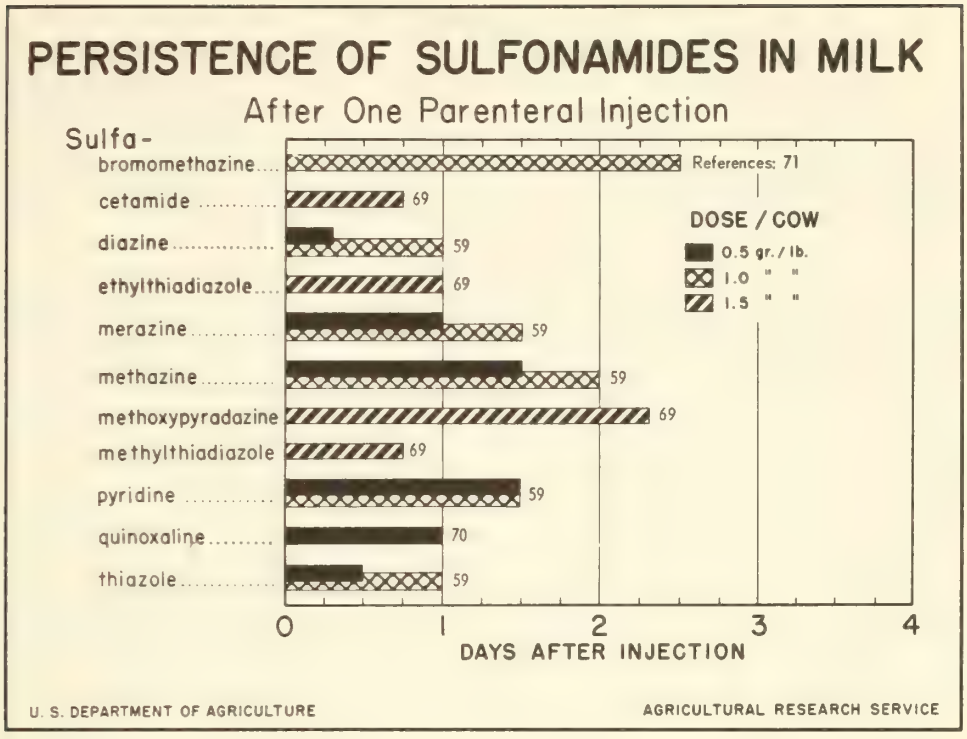
Sulfonamides--Figure 7 shows a comparison of various sulfonamides given parenterally at different dose levels. The persistence of the sulfonamides in milk after one injection depends upon the dose and the type of sulfonamide. The two sulfonamides not commonly used, sulfabromomethazine and sulfamethoxypyradazine, lasted the longest. Therefore, we might conclude that the more commonly used sulfonamides do not persist in the milk any longer than 48 hours after a single injection. The only exception is sulfamethazine which lasted 48 hours but was not tested at the dose of 1.5 gr./lb. of body weight. The maximal concentrations of the sulfonamides when last detected in the milk ranged from 0.1 to 1.2 mg./100 ml. of milk (59,69,70,71).

THE ORAL ADMINISTRATION OF ANTIBIOTICS AND SULFONAMIDES

Antibiotics--Mastitis is not treated by the oral administration of antibiotics, although some of the antibiotics are fed at low levels to control bloat and prevent infections (according to some advertisements) and could appear in the milk. Skaggs and Miller (65) reported a level of 0.05 u./ml. of milk when two cows were fed daily as little as 177.8 mg. (173,400 units) of procaine penicillin in the concentrate feed. These levels increased to 0.10 and 0.15 unit when the daily dose was 277.8 mg. (270,500 units). This work has not been confirmed in recent experiments since two cows were fed one daily dose of 1 million units of potassium penicillin for 10 days without any penicillin being detected in the milk (82). Negative results were also obtained when two cows were fed 5 million units daily for 5 days with each of two daily feedings containing half the dose (82). Further work is needed to clarify these discrepancies. In addition, bacitracin did not appear in the milk when 0.5 gm. was fed once a day in the grain for 10 days, nor did streptomycin when 0.5 gm. was fed twice a day for 5 days (82).

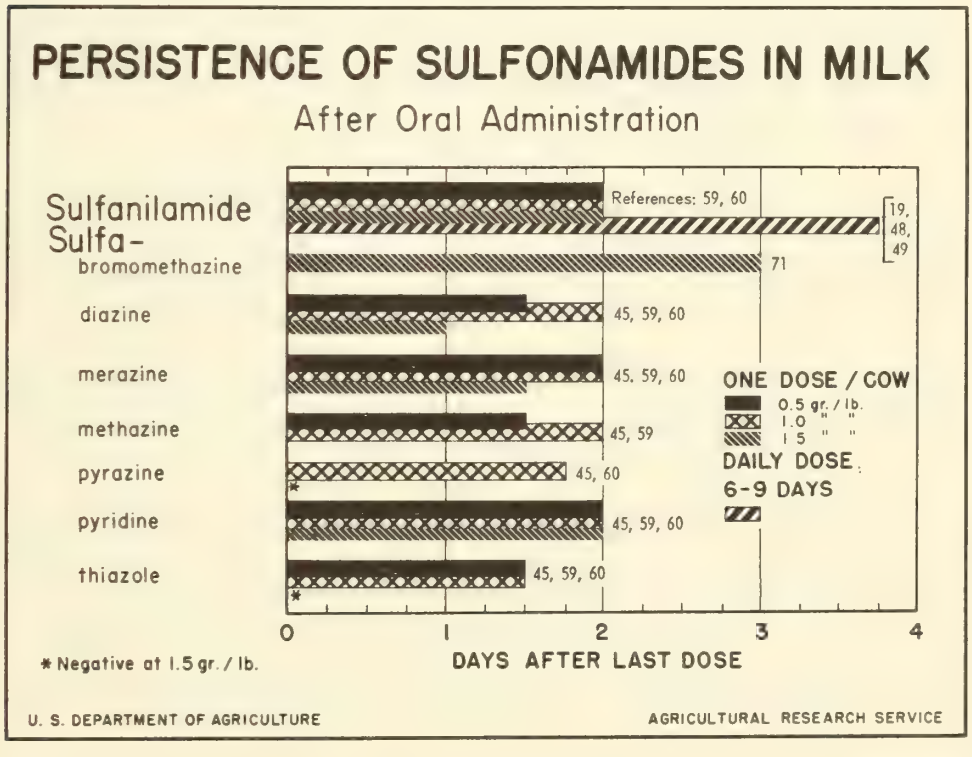
Loosli and Warner (44) first reported that no Aureomycin was found in the milk of cows that had received 700 mg. per day for 10 days. Martin, et al. (46) also had negative results when they fed Aureomycin daily at the rate of 32 mg./100 lbs. of body weight or an average of 390 mg. per cow. Since then Henderson, et al. (25) and Shor, et al. (62) have found Aureomycin in the milk when cows were fed at levels of 0.5 mg./lb. of body weight per day and higher. No Aureomycin was found when a dose of 0.1 mg./lb. of body weight was fed daily. Using the data of Shor et. al. (62), it was calculated that during the feeding of Aureomycin at the level of 0.5 mg./lb., the average concentration was 0.045, μg./ml. of milk, which increased to 0.083, μg./ml. when 1.0 mg./lb. was fed.

Sulfonamides--Figure 8 shows the persistence of sulfonamides in the milk after oral administration. Sulfabromomethazine persisted for 3 days in the milk after a single dose; however, 2 days was the longest time any of the more commonly used sulfonamides lasted. The size of the dose does not seem to have any marked effect on their persistence. Although data are only presented for sulfanilamide, it would appear that dosing for several days may cause these drugs to persist longer after the last dose is given. The maximal time that sulfanilamide persisted after one dose was 48 hours, whereas when cows were given the drug for 6 consecutive days it persisted in the milk for 90 hours after the last dose (48,49). On the other hand, Gildow, et al. (19) fed cows sulfanilamide at the rate of 10 to 15 gm./100 lb. of body weight daily for 9 days and found that the drug disappeared from the blood and milk within 48 hours after feeding was stopped.



BN-10705-X

Figure 7.--Parenteral injection: The maximal time various sulfonamides persisted in milk when different doses were used.



BN-10710-X

Figure 8.--Oral administration: The maximal time various sulfonamides persisted in milk when different doses were used.

DISCUSSION

Antibiotics and sulfonamides can enter the milk supply by the following routes: (1) The treatment of cows for mastitis with intramammary preparations; (2) the parenteral injection of these drugs for the treatment of mastitis or other diseases; (3) the oral administration of antibiotics in feeds at levels considerably higher than are not recommended, or the oral administration of sulfonamides for the treatment of diseases; and (4) illegal addition of antibiotics to milk for the preservative effect.

Many veterinarians and others feel that over-the-counter sale of antibiotic preparations has been the greatest contributing factor to the problem of antibiotics in milk (7,21). Although this may be true, the direct sale of antibiotics to farmers is not practiced in the Scandinavian and European countries; yet antibiotics in milk have been a problem to the cheese industry in these countries. As pointed out by Welch, et al. (80), the great bulk of the milk on the American market that contains antibiotic residues results from the complete disregard of the warning on the label of each mastitis preparation to discard milk from treated quarters for at least 72 hours after the last treatment.

The fact that antibiotics can persist in the udder longer than 72 hours indicates that a dairyman could adhere to the recommended use of the antibiotic preparations and yet inadvertently contaminate the milk supply. The small amounts present on the fourth day after treatment would probably not be detected if only a few quarters were treated at any one time. If a large number of quarters were treated, as is often done to eradicate Streptococcus agalactiae infection from a herd, there would be a greater possibility of having detectable levels in the milk from that herd on the fourth day after treatment.

The rapid test for penicillin used by dairy plants and F.D.A. for checking interstate shipments of milk is sensitive enough to detect 0.05 u./ml. of milk. Penicillin levels greater than this amount can cause failures in the manufacture of various dairy products (34). If we consider 0.23 u./ml. of milk as the average--median of the range of units in figure 3--amount of penicillin that could be present on the fourth day after treatment of a quarter, then approximately 22 percent of the quarters in any one herd would have to be excreting penicillin on the fourth day before it could be detected in the herd milk by this test. If the overnight test for penicillin is used to check the milk, then only about 2 percent of the quarters would have to be excreting penicillin on the fourth day, since this test is about 10 times more sensitive than the rapid test.

Kelley and Kanegis (40) treated six quarters with 426 mg. of Aureomycin in ointment and found the average concentration was 0.32 μg./ml. of milk 96 hours after treatment. The test was sensitive to 0.025, μg./ml. of milk. Consequently, about 8 percent of the quarters in any one herd would have to be treated at one time before Aureomycin could be detected in the herd milk 4 days after treatment.

These mathematical manipulations are not presented to indicate that more than a 3-day persistence of antibiotics in milk is the cause of our present problem. We only wish to emphasize a potential problem. As more dairy plants test milk for penicillin and other antibiotics, these exceptional cases will be found and should be recognized as such.

Products that provide therapeutic levels of penicillin in the animal for long periods of time have always been desired by veterinarians because of the expense and inconvenience of repeated injections at frequent intervals. These products have been supplied by the pharmaceutical companies. From the viewpoint of treating mastitis and other diseases in dairy cattle, this is desirable; however, from the viewpoint of residues in milk, it is undesirable. The long-acting salts of penicillin, such as benzathine, are not used in intramammary preparations in this country. Apparently, no difference in persistence is shown by the presently used sodium, potassium, or procaine salts of penicillin (26,37). Therefore, a reevaluation of the oil-base vehicles might be indicated since most contain aluminum monostearate, which may have a prolonging effect on the persistence of penicillin in milk (37,63). Unless we are only interested in eliminating penicillin from milk, the substitution of other antibiotics is not the answer. Broad spectrum

antibiotics, such as Aureomycin or Terramycin, would have to be used; these also can persist in milk longer than 3 days after intramammary infusion.

In addition, we should question the use of some of the penicillin products available for intramuscular injection of dairy cattle. Procaine penicillin G in oil and benzathine penicillin G and V in aqueous suspension may persist in milk for 6 days or longer after intramuscular injection with doses of 5,000 u./lb. of body weight (5 to 8 million units per cow) or higher. It would seem best, therefore, not to use these products in dairy cattle. Available data indicate that the sodium, potassium, diethylaminoethylester hydriodide, and procaine salts of penicillin G in aqueous suspension may last from 1 to 3-1/4 days at maximal doses of 10,000 u./lb. of body weight. Aureomycin, Terramycin, tetracycline, streptomycin, and most sulfonamides do not persist in milk longer than 2 days after parenteral injection of recommended doses. Consequently, by education of the veterinarian and farmer these products can be used and not be a source of contamination of milk.

The commonly used sulfonamides persist in milk no longer than 2 days after one oral dose, with the exception of sulfabromoethazine (3 days). In most instances, sulfonamides are given orally for several days, or one intravenous injection is given followed by several oral doses. More information, therefore, is needed on the persistence of sulfonamides in milk after repeated dosing.

We hope that this limited review will point the way for further research on the persistence of antibiotics and sulfonamides in milk.

REFERENCES

(1) Anonymous. 1960. Antibiotic residues in milk. Animal Disease Trends. 7(3): 1-2.

(2) Barker, C. A. V., and Dussault, H. P. 1945. Observations on the permeability of the lactating bovine mammary glands to penicillin. Canad. J. Comp. Med. 9: 332-335.

(3) Barnes, L. E. 1956. Oxytetracycline in bovine mastitis. II. Milk levels following local and parenteral administration. Amer. J. Vet. Res. 17: 18-23.

(4) Bauer, H., and Gunderson, M. F. 1938. Para-aminosulfamidobenzene in amniotic fluid and placental blood. J.A.V.M.A. 93: 118.

(5) Bell, W. B., Flora, C. C., Reaves, P. M., and Holdaway, C. W. 1951. Aureomycin concentration in milk following intramammary infusion and its effect on starter activity. J. Dairy Sci. 34: 675-679.

(6) Bierlein, K. J. 1956. Repeated anaphylactic reactions in a patient highly sensitized to penicillin. Ann. Allergy 14: 35-40.

(7) Boland, H. 1957. The new frontier in quality control. American Milk Review, Sept.

(8) Boulton, W. D., Frazer, J. M., Cady, J. H., and Waller, E. F. 1949. Procaine penicillin G levels in udder during treatment of chronic mastitis. Vet. Med. 44: 244-245.

(9) Bradfield, A., Resi, L. A., and Johnstone, D. B. 1952. The presence of Aureomycin in milk and its effect on cheese making and starter activity. J. Dairy Sci. 35: 51-58.

(10) Carmichael, J. 1949. The persistence of penicillin in milk. Vet. Rec. 61: 886.

(11) Coleman, M., and Siegel, B. B. 1955. Studies in penicillin hypersensitivity. II. The significance of penicillin as a contaminant. J. Allergy 26: 253-261.

(12) Doan, F. J. 1956. Antibiotics in milk from farms. J. Dairy Sci. 39: 1766-1767.

(13) Downing, H. E., Hardie, W. B., Hawley, G. E., and Price, K. E. 1954-1955. Tetracycline in veterinary medicine. Antibiotics Annual 312-317.

(14) Drury, A. R. 1952. Evaluation of neomycin sulfate in treatment of bovine mastitis. Vet. Med. 47: 407-411.

(15) Edwards, S. J., and Haskins, M. D. 1953. The determination of antibiotic levels in blood and in milk following parenteral and intramammary injections. J. Comp. Path. & Therap. 63: 53-67.

(16) Foley, E. J., and Byrne, J. V. 1949. Observations on the treatment of Streptococcus agalactiae and Staphylococcus aureus udder infections with mixture of streptomycin and penicillin. Cornell Vet. 39: 243-248.

(17) Foley, E. J., Stults, A. W., Lee, S. W., and Byrne, J. V. 1949. Studies on vehicles for sustaining penicillin levels in the bovine mammary gland. Am. J. Vet. Res. 10: 66-70.

(18) Freedman, M. L., and Meara, P. J. 1959. Antibiotic contamination of milk supplies as a veterinary and public health program. South African Practitioner. 5. Nos. 1, 2, 3 and 4.

(19) Gildow, E. M., Fourt, D. L., and Shaw, A. O. 1938. Sulfanilamide in the treatment of streptococcic mastitis. J. Dairy Sci. 21:759-766.

(20) Grove, D. C. 1959. Prevention of antibiotics in milk--present status. J. Dairy Sci. 42: 199-201.

(21) Guss, S. B. 1958. Survey of Pennsylvania veterinarians on mastitis and the antibiotics problem. Vet. Med. 53: 50-52.

(22) Hammond, P. B. 1953. Dihydrostreptomycin dose-serum level relationships in cattle. J.A.V.M.A. 122: 203-206.

(23) Hansen, H. C., Wiggins, G. E., and Boyd, J. C. 1950. Modern methods of mastitis treatment cause trouble in the manufacture of fermented dairy products. Proc. 31st annual meeting Western Div. Amer. Dairy Sci. Assoc. 67-74.

(24) Hawley, G. E., Downing, H. E., Zolli, Z., Sacchi, E. M., English, A. R., and Peterson, E. H. 1954-1955. Oxytetracycline in mastitis. Antibiotics Annual 329-335.

(25) Henderson, B. W., Cobble, J. W., Easterbrooks, H. L. 1957. The recovery of milk and blood levels of Aureomycin when fed to lactating dairy cows. J. Dairy Sci. 40: 617.

(26) Hignett, S. L. 1950. Persistence of penicillin in the bovine udder. Vet. Rec. 62: 37-38.

(27) Hollister, C. J., Huebner, R. A., Boucher, W. B., and DeMott, T. 1957. Parenteral benzathine penicillin V in cattle. Am. J. Vet. Res. 18: 584-586.

(28) Hollister, C. J., Huebner, R. A., Boucher, W. B., and DeMott, T. 1959. Parenteral benzathine penicillin V in bovine mastitis. Am. J. Vet. Res. 20: 287-296.

(29) Hovmand, H. C., and Overby, A. J. 1955. Elimination and bacteriological effect of "tardocillin" during a collective campaign of streptococcal mastitis. (In Danish). Nord. Vet.-Med. 7: 833-842.

(30) Hughes, D. L., Christie, G. J., and Farmer, R. K. 1950. Procaine penicillin and interrupted penicillin treatment in Streptococcus agalactiae mastitis. Vet. Rec. 62: 11-14.

(31) Hughes, D. L., and Farmer, R. K. 1950. Studies on strepotomycin in relation to its possible uses in veterinary practice. Vet. Rec. 62: 265-270.

(32) Hunter, G. J. E. 1949. The effect of penicillin in milk on the manufacture of cheddar cheese. J. Dairy Res. 16: 235-241.

(33) Jackson, W. F., and Bryan, C. S. 1950. Penicillin milk levels in cows following intramammary administration. Vet. Med. 45: 395-399.

(34) Jepsen, A., and Overby, A. 1951. The disturbing effects of penicillin milk in the dairy industry and proposals of some technical methods for the elimination of failures; studies on the inactivation of penicillin by means of penicillinase. Nederlands Milk--enzuiveltijdschr. 5: 157-185.

(35) Jester, W. R., Wright, W. W., and Welch, H. 1959. Antibiotics in fluid milk. Fourth Nationwide Survey. Antib. & Chemo. 9: 393-397.

(36) Jorgensen, A. 1951. On souring difficulties with special reference to the effect of penicillin-containing milk. (In Danish) Nord. Vet.--Med. 3: 203-246.

(37) Juncher, H., Magnusson, I., and Romer, O. 1950. Measurements of penicillin content of milk after intramammary infusion of penicillin preparations. (In Danish) Nord. Vet.-Med. 2: 765-788.

(38) Kanegis, L. A., Tonelli, G., and Falk, H. 1953. Aureomycin milk and serum concentrations in dairy cows. J. Dairy Sci. 36: 152-155.

(39) Kelley, W. R., and Bell, F. R. 1947. Some observations on the pharmacology of penicillin in cattle. Vet. Rec. 59: 591-594.

(40) Kelley, R. G., and Kanegis, L. A. 1960. Personal Communication. American Cyanamid Company, Pearl River, New York

(41) Krienke, W. A. 1950. Drugs, mastitis and acid starters. Am. Milk Rev. 12(6): 54-56.

(42) Langer, P. H., Burkhart, R. L., Schroeder, C. R., and Welsh, M. 1948. Sulfamethazine blood and milk concentrations in dairy cows. J. Dairy Sci. 31: 103-109.

(43) Larsen, P. H., and Gilman, H. L. 1950. Aureomycin as a treatment of acute brucellosis of cattle. Cornell Vet. 40: 259-272.

(44) Loosli, J. K., and Warner, R. G. 1952. Antibiotics for dairy animals. Farm Research. 18: No. 3.

(45) Maclay, M. H., and Slavin, G. 1947. Sulphonamide investigations. I. The absorption and excretion in dairy cows of sulphapyridine, sulphamezathine, sulphamerazine, sulphadiazine, sulphathiazole and sulphapyrazine. Vet. Rec. 59: 313-317.

(46) Martin, W. H., Claydon, T. J., and Bartley, E. E. 1955. Aureomycin content, bacterial development, starter activity, and cheese quality of milk from cows fed on aureomycin supplement. J. Dairy Sci. 38: 47-52.

(47) McCulloch, E. C., Kiser, J. S., and Migaki, H. 1949. Aureomycin in the treatment of staphylococcic mastitis in cows. Vet. Med. 44: 253-258.

(48) Miller, W. T., Mingle, C. K., Murdock, F. M., and Heishman, J. O. 1939. The concentration of sulfanilamide in the blood and milk of cattle and its effect on Brucella abortus and streptococcal infections of the bovine udder. J.A.V.M.A. 94: 161-171.

(49) Miller, W. T., Murdock, F. M., and Heishman, J. O. 1939. Second report on the use of large doses of sulfanilamide in the treatment of chronic streptococcal mastitis. J.A.V.M.A. 95: 140-145.

(50) Murname, D. 1945. A preliminary report on the treatment of clinical and subclinical streptococcal and staphylococcal infections of the bovine udder with penicillin. Aust. Vet. Jour. 21: 82-90.

(51) Overby, A. J. 1952. The effect of various antibiotics in milk following intramammary infusion. Nord. Vet.-Med. 4: 993-1004.

(52) Overby, A. J. 1954. Antibiotics in milk. Commonwealth Bur. Dairy Sci., Dairy Sci. Abstracts 16: 1-24.

(53) Randall, W. A., Durbin C. G., Wilner, J., and Collins, J. H. 1953-54. Antibiotic concentration and duration in animal tissue and body fluids. I. Blood serum and milk of cows. Antibiotics Annual: 421-427.

(54) Sadek, S. E. 1954. Penicillin concentration in bovine blood and milk after intramuscular injection and its application in the treatment of mastitis. J.A.V.M.A. 125: 387-390.

(55) Schalm, O. W., and Casselberry, N. H. 1946. Treatment of Streptococcus agalactiae infections with intramammary infusions of penicillin. J.A.V.M.A. 109: 470-477.

(56) Schalm, O. W., Bankowski, R. A., Ormsbee, R. W., and Browne, T. W. 1949. Effect of sulfamethazine on certain infections of the bovine mammary gland. Am. J. Vet. Res. 10: 56-62.

(57) Schipper, I. A., and Petersen, W. E. 1952. Milk, blood and urine concentrations of aureomycin after intramammary infusions and intravenous administration. Vet. Med. 47: 367-371.

(58) Schipper, I. A., and Petersen, W. E. 1953. Milk, blood, and urine concentrations of terramycin following intravenous, intramammary, and intramuscular administration. Vet. Med. 48: 315-318.

(59) Schipper, I. A., and Eveleth, D. F. 1959. Rates and routes of sulfonamide excretion in the cow. I. Milk levels following single intravenous and oral administrations. Am. J. Vet. Res. 20: 714-717.

(60) Schuhardt, V. T., Carroll, T. B., Rode, L. J., and Lacy, H. 1948. The permeability of the lactating bovine mammary gland to sulfonamides. Am. J. Vet. Res. 9: 144-146.

(61) Seeley, H. W., Jr., Anderson, E. O., Plastridge, W. N., and Pearson, P. 1945. Non-permeability of the lactating bovine mammary gland to penicillin. Science 102: 44-45.

(62) Shor, A. L., Johnson, W. P., and Abbey, A. 1959. Effects of various amounts of chlortetracycline in the rations of lactating dairy cattle. J. Dairy Sci. 42: 1203-1208.

(63) Simensen, B. 1959. Penicillin preparations for intramammary application. Nord. Vet.-Med. 11: 523-539.

(64) Simon, J., Spencer, G. R., and Schenk, M. 1951. Penicillin in oil retention vehicles in the treatment of streptococcic mastitis. Vet. Med. 46: 387-389.

(65) Skaggs, S. R., and Miller, D. D. 1959. Penicillin levels in milk from lactating cows fed various amounts of procaine penicillin. J. Dairy Sci. 42: 1742.

(66) Slavin, G. 1946. Penicillin concentration in the blood and milk of bovines, Proc. Roy. Soc. Med. 39: 793-795.

(67) Smith, C. R., Petersen, W. E., and Brown, R. W. 1948. Effect of infused streptomycin in the mammary gland. Proc. Soc. Exper. Biol. & Med. 68: 216-219.

(68) Storrs, F. C., and Hiett-Brown, W. 1954. The incidence of penicillin in milk supplies. J. Dairy Res. 21: 337-341.

(69) Stowe, C. M., Hammond, P. B., Aronson, A. L., and Kriewaldt, F. H. 1957. A survey of some of the pharmacological properties of four sulfonamides in dairy cattle. Cornell Vet. 47: 469-479.

(70) Stowe, C. M., Pallesen, D., and Hartman, W. 1957. Studies on the pharmacology of sulfaquinoxaline in dairy cattle. Am. J. Vet. Res. 18: 511-518.

(71) Stowe, C. M., Aronson, A. L., and Johnson, K. 1958. The pharmacology of sulfabromomethazine, a new long-acting sulfonamide, in cattle. Am. J. Vet. Res. 19: 345-353.

(72) Thorp, W. T. S., Uhrik, I. J., and Straley, E. J. 1947. Concentrations of penicillin in the bovine mammary gland following infusion and the penicillin tolerance of certain streptococci. Am. J. Vet. Res. 8: 157-165.

(73) Trussell, P. C., and Stevenson, W. G. 1949. The penicillin concentrations in milk following insertion of penicillin bougies into the bovine udder. Canad. J. Comp. Med. 13: 127-129.

(74) Trussell, P. C., and Stevenson, W. G. 1949. Streptomycin concentrations in milk following insertion of streptomycin bougies into the bovine udder. Canad. J. Comp. Med. 13: 209-212.

(75) Uvarov, O., and Muggleton, P. W. 1954. Penicillin levels in cow's milk after intramammary infusion of procaine penicillin. Vet. Rec. 66: 200-202.

(76) Vickers, H. R., Bagratuni, L., and Alexander, S. 1958. Dermatitis caused by penicillin in milk. Lancet 1(7016): 351-352.

(77) Watts, P. S., and McLeod, D. M. 1946. The estimation of penicillin in blood serum and milk of bovines after intramuscular injection. J. Comp. Path. & Therap. 56: 170-179.

(78) Weirether, F. J., Jasper, D. E., and Petersen, W. E. 1945. Effect of infused penicillin in the bovine mammary gland. Proc. Soc. Exper. Biol. & Med. 59: 282-286.

(79) Welch, H., Jester, W. R., and Burton, J. M. 1955. Antibiotics in fluid milk. Antib. and Chemo. 5: 571-573.

(80) Welch, H., Jester, W. R., and Burton, J. M. 1956. Antibiotics in fluid market milk. Third Nationwide Survey. Antib. & Chemo. 6: 369-374.

(81) Welsh, M., Langer, P. H., Burkhart, R. L., and Schroeder, C. R. 1948. Penicillin blood and milk concentrations in the normal cow following parenteral administration. Sci. 108: 185-187.

(82) Wright, W. W. 1960. Antibiotics Division, Food and Drug Administration, Dept. Health, Education and Welfare. Personal Communication.

(83) Zimmerman, M. C. 1959. Chronic penicillin urticaria from dairy products, proved by penicillinase cures. Archives of Dermatology 79: 1-6.

(84) Zweig, J. 1949. Procaine penicillin G in the bovine udder. Vet. Rec. 61: 811-813.

ANTIBIOTICS, HORMONES, AND TRANQUILIZERS IN ANIMALS

J. F. Sykes, J. W. Thomas, J. Bitman, and C. F. Winchester [1]

INTRODUCTION

The use of antibiotics, hormones, and tranquilizers as feed additives or by other forms of administration for improving efficiency of livestock and poultry production introduces several problems beyond an evaluation of their usefulness for the purpose proposed. In addition to the possibility that these substances may accumulate in the tissues and animal products used for human consumption, there exists the possibility that the metabolic products of these compounds may also persist and produce harmful effects when consumed. The problem of exposure of other animals or foods to the excreta from treated animals with possible harmful results to both humans and animals must also be considered. A detailed knowledge of absorption, excretion, metabolism, and stability of these materials would seem to be a prerequisite for estimating the problems that might arise in their use or for the solution of these problems as they arise.

In this presentation, we have attempted to review to what extent this area of work has been considered in the research associated with the use of these compounds and to compile information on their disposal and metabolism that would provide guidelines for the nature of future investigations. We have restricted our review to the antibiotics, hormones, and tranquilizers, rather than try to examine all the possible compounds within each class. Our discussion is also restricted to those compounds in general use at present or those proposed for use by livestock.

The problem of obtaining precise and meaningful information in these areas of work is very great. Most of these compounds are complex organic molecules. While tests and analytical methods exist for these compounds, they are frequently not specific for a particular material. This imposes the problem of developing adequate extraction and purification procedures before reliance can be placed on the analytical results. Biological assay methods are useful in tracing the primary activity of interest but give little precise information on the nature of metabolic transformations. These methods in general suffer from lack of precision, particularly when dealing with micro quantities in tissues and excreta.

ANTIBIOTICS

Absorption

When administered by any parenteral route the antibiotics are all quickly absorbed and translocated from the site of injection. When given orally some are absorbed quickly

---

[1] Veterinarian (Nutrition) and Leader, Nutrition and Physiological Investigations; Dairy Husbandman; Chemist, Dairy Cattle Research Branch; and Animal Husbandman (Nutrition), respectively, Animal Husbandry Research Division, Agricultural Research Service, U. S. Department of Agriculture.

while some are absorbed in very small quantities, if at all. Bacitracin, streptomycin, neomycin, and polymyxin are members of the latter group, while penicillin, chloramphenicol, carbomycin, tetracycline, and related cyclines are all rapidly absorbed from the gastrointestinal (G.I.) tract. Absorption apparently occurs from all sections of the gastrointestinal tract. The last-named group of antibiotics are also absorbed when applied topically and by lung tissue from aerosol or fine dusts.

In general and within broad limits the larger the continual oral dose the higher the concentration in the blood serum and other tissues. However, such a relationship is commonly not found after the administration of a single dose. Maximum concentration in blood serum occurs about 1 to 3 hours after ingestion in animals with simple stomachs, but requires 8 hours in calves. The maximum blood concentration usually occurs within 1 hour after intravenous or intramuscular administration (I.M.), although there is a large difference among species in this respect. The absorbable antibiotics can persist in the bloodstream and other tissues for at least 24 hours after ingestion in simple stomached animals and up to 7 days in the serum, liver, and kidney of calves. The length of persistence depends on the size of the oral dose (1,2,3,4,5,6,7).

The extent and rate of absorption are influenced by many factors. Most of these have been studied using humans, a few using laboratory animals (rat, rabbit, chicken, dog, and cat), but none of these factors have been studied using large farm animals. In most studies the presence of food has increased the extent of absorption although with some antibiotics the opposite has been found (5). Concurrent administration of aluminum hydroxide jels and other antiacids has been shown to reduce the absorption of the cycline antibiotics. Erythromycin is destroyed by gastric secretions and must be given in protected tablet form.

The amount of calcium in the diet affected the extent of absorption of oxy- and chlortetracycline. When chickens were given this antibiotic at 200 gm./ton of feed ($\pm$ 20 mg./kg. body weight), the concentration in the blood plasma on the low calcium diet was double that obtained on a regular diet (0.10 vs. 0.20 μg. chlortetracycline per ml. plasma). Similar results were obtained at dietary antibiotic levels two and four times that amount. The concentration of chlortetracycline in the feces was also less when on the low calcium diet (6,7,8). Similar results have been obtained in swine where diets low in calcium (0.06 percent) doubled the chlortetracycline levels in blood plasma (0.54 to 1.06 μg. chlortetracycline/ml.) compared to normal diets containing 0.70 percent calcium (9). The addition of phosphorus to diets also increased the absorption of chlortetracycline (6, 10).

Simultaneous administration of citric acid has increased blood levels of the cyclines in rats, guinea pigs, dogs, chickens, and pigs but not in man (2,5,6,7,10). In view of all the animal data showing this effect the early work with humans needs to be repeated. Other effective adjuvants were trisodium citrate, malic, tartaric, malonic, pyruvic, and lactic acids, monosodium phosphate, tricarballyic acid, terephthalic acid, camphoric acid, and ethylenediamine tetraacetic acid (EDTA). These compounds increased blood-serum levels of chlortetracycline by 47 to 114 percent in chickens (6). These and other less effective adjuvants all are capable of complexing calcium ions.

The simultaneous administration of various multivalent metallic ions with the cyclines has lowered their blood levels (6, 11). The substitution of calcium sulfate for calcium carbonate in poultry and swine diets resulted in increased levels of chlortetracycline when the antibiotic was fed (6,7,9). Diets low in calcium are now in use to help increase the effectiveness of the cyclines in treatment of poultry diseases.

Additional but more precise information on factors affecting absorption have been obtained by the use of ligated intestinal loops where the antibiotic, adjuvant, and metallic ions were injected into the loop. Subsequent levels of the antibiotic in serum, liver, kidney, and loop contents were used to estimate extent of absorption (10). Total absorption of oxytetracycline from the loop varied from 20 to 45 percent with an average of 30.5 percent.

Penicillin is capable of forming many types of esters and salts which alter the extent and rate of absorption. Penicillin V (phenoxymethylpenicillin) has given higher blood and tissue levels than potassium G (5).

Accumulation in Tissues

Before discussing distribution and accumulation in body fluids and tissues, a general statement on excretion appears desirable. After oral administration, by far the most extensive pathway of excretion is in the feces. After intravenous or intramuscular injection the largest amount is excreted in the urine with usually only small amounts appearing in the feces. In both methods of administration the bile contains the antibiotic and resorption from the gastrointestinal tract occurs. The concentration in urine and bile is usually 10 to 100 times higher than the concentration in blood serum.

Practically all data obtained to date on the distribution and accumulation of antibiotics have been determined by microbiological techniques wherein the material under assay must be active against the test organism employed. One must keep in mind that this may or may not give a complete picture of the fate of the antibiotic.

When given in adequate dosage levels, the antibiotic can be found in most of the body fluids and tissues such as peritoneal, pericardial, pleural, and ascitic fluids, kidney, liver, spleen, intestine, muscle, brain, cerebrospinal fluid (C.S.F.), all parts of the eye, bile, urine, milk, saliva, and in the fetus and its circulation.

Once absorbed most antibiotics appear to follow a diffusion gradient and appear in all the tissues. However, there are some exceptions to this generalization--neomycin is apparently not found in C.S.F.; bacitracin in C.S.F. is not found in man but found in dogs nor is it found in the anterior part of the eye; erythromycin is not found in the brain and only in traces in C.S.F. (2,5,7).

Most studies on the concentration of the antibiotic in blood serum have been obtained on humans. The results for any one species cannot be applied to another specie without serious reservations. The administration of a constant oral dosage produces serum levels in swine that are double those found in poultry (7). Similarly, with various cycline antibiotics the blood level in rats was found to be 10 times higher than those in rabbits (11). An intramuscular dose of 15 mg./kg. produced serum levels in dogs that were 2 to 5 times higher than in rabbits.

There was a large difference among individuals receiving the same dosage and it was not influenced by exercise (2,5,7). Inflammation will permit the antibiotic to diffuse into organs where it otherwise is not usually found (5).

On a constant dosage the serum concentration may be different with animals of different ages. In chickens when given the antibiotic as a constant percent of the diet the difference in blood levels at different ages were correlated with the feed intake per pound of body weight (6,7). In humans the renal clearance of bicillin (a penicillin ester) was found to decrease as age increased.

Blood levels of chlortetracycline were not affected by the presence of milk products in the diet except as they altered calcium content, nor by the addition of 2 to 8 percent fat, but the presence of vitamin D in the diet increased the blood levels of this antibiotic (7).

The amount of antibiotic in the tissues is a function of amount and length of administration as well as a function of route of administration as mentioned previously.

When chlortetracycline was given to calves for 3 months at the approximate rate of 50 mg./day it was found in the bile, feces, urine, liver, kidney, and blood. None was found in other tissues such as muscle, thymus, and similar glands. Oral administration resulted

in larger concentrations (12 times larger) in the feces and in all sections of the gastrointestinal tract than did intramuscular injection of the same dose when only very small amounts were found in the lower intestine and feces. None was found in the rumen after intramuscular administration. Conversely after intramuscular injection, urine levels were four to five times that obtained after oral administration. Similarly, liver and kidney had measurable amounts but only traces after oral administration. With both methods of administration the bile contained the same concentration (0.6 μg./ml.), while only traces were found in the blood (12). When a single dose of 125 mg. was given, appreciable levels were found in the blood, liver, and kidney for 7 days (7). Three days after withdrawal following long-term ingestion of about 500 mg./day/calf the liver contained 0.02 μg. chlortetracycline/gm. but none was found in the blood or kidney (7). Information with other species indicates that the liver may act as a place of storage for the cycline antibiotics (2,5,18,30).

One intramuscular injection of a mixture of benzathine, procaine, and potassium salts of penicillin G (12,500 to 50,000 units/kg.) to dairy steers produced a maximum blood level (2-9 μg./ml.) within 1/2 hour and measurable amounts (0.02 to 0.05 μg./ml.) were found in the serum for 6 days (13).

When 130 to 700 mg. of the tetracyclines were ingested per day by lactating cows, the milk produced did not alter the growth of several sensitive cheese starter cultures which indicated that very little of the antibiotic was secreted into the milk (14, 15, 16). When 0.1 mg. chlortetracycline/lb. body weight (100 mg./day) or when 320 mg. per day was ingested, no antibiotic was found in the milk. However, when 0.5 or 1.0 mg./lb. (500 to over 1,000 mg./day/cow) was ingested, small amounts (0.01 to 0.06 μg./ml.) of the antibiotic were found in the milk 24 hours after ingestion. With continued administration the concentration of chlortetracycline in the milk increased for a few days to levels of 0.03 to 0.20 μg./ml., and then stabilized at levels of 0.00 to 0.09 μg./ml. No antibiotic was found in the milk 24 hours after removal of chlortetracycline from the diet (16,17).

When fed to dairy cattle, the recommended level is 0.1 mg. chlortetracycline/lb. body weight or about 100 mg./cow/day. At this rate none has been detected in the milk by several investigators. No information on the content of chlortetracycline in the feces, urine, or tissues of these cows is available but based on the reported results with calves (7,12) very little, if any, would be expected in tissues of cows receiving the recommended level.

There was no detectable amount of antibiotic in any internal organ or muscle of pigs fed the antibiotic from weaning to market weight at the rate of 5 or 83.5 gm. bacitracin/ton of feed. The same result applied to penicillin fed at 1 or 16.7 gm./ton of feed or with chlortetracycline fed at 10 gm./ton (19). The usual level of feeding to swine is about 10 gm./ton.

When oxytetracycline at various levels was continuously fed to pigs, none was found in any tissue until the level of 200 gm./ton was reached when low concentrations (0.2 to 0.7 μg./gm.) were found in the kidney and intestine, respectively. At levels of 1,000 to 2,500 gm./ton of feed it appeared in all tissues, but the intestine contained 4 to 5 times that found in the liver and kidney (1.0 to 5.0 μg./gm.). The muscle contained 1.1 to 1.3 μg. oxytetracycline/gm. in each case (20).

Young baby pigs fed a diet containing 200 gm. chlortetracycline per ton of feed had this antibiotic in their blood serum at levels of 0.1 to 0.5 μg./ml., but none was present after the antibiotic was withdrawn for 2 days (21).

In none of the above trials is the actual amount of the antibiotic consumed stated.

Concentration of chlortetracycline was approximately the same in fat as in lean muscle tissue (6).

Chlor-, oxy-, and tetracycline appeared in the blood serum of young chickens when fed at the rate of 50 gm. antibiotic per ton of feed. However, even at the level of 200

gm./ton (approximately 200 p.p.m.) not every chick had detectable amounts of the antibiotic in their blood. One day after cessation of antibiotic feeding the serum contained no antibiotic when the feeding levels had been 200 to 1,000 gm./ton (approximately 200-1,000 p.p.m.). When the dietary level was 5,000 gm./ton, some was found in the serum until after the third day (18).

The antibiotic was found in the liver when the diet contained 50 p.p.m. chlortetracycline or 200 gm. oxytetracycline per ton and in the muscle at dietary levels of 50 p.p.m. and 1,000 gm./ton, respectively. The tissue content actually decreased as time on the diet increased (18,20). These differences noted may be due to absorption, assay method, or experimental technique. It disappeared rapidly from the tissues when the antibiotic was withdrawn from the diet. The levels in the liver and muscle were practically zero one day after cessation of feeding levels of 1,000 or 2,500 gm. oxytetracycline/ton feed. At higher levels it persisted in the kidney and intestine for about 3 days. Twenty-four hours after cessation of feeding 200 p.p.m. chlortetracycline in the diet 94 percent of the antibiotic in the muscle, liver, and blood had been excreted (6,18,20,21).

Chlortetracycline feeding at the rate of 200 p.p.m. to laying hens produced low amounts (0 to 0.1 μg./gm.) of the antibiotic in the eggs. Here also the concentration in the eggs decreased with increasing length of time that the antibiotic was fed at this level as well as at the 2,000 p.p.m. level of feeding. After withdrawal for 1 week none was found in the eggs when the feeding level was 200 p.p.m. and only small amounts (0 to 0.13 μg./gm.) persisted for 2 weeks at the 2,000 p.p.m. feeding level (18).

The adjuvants also increase the concentration of chlortetracycline in the tissues, but no information is available on the amount in eggs.

The usual feeding level to promote growth in poultry is 4 to 10 gm. antibiotic per ton of feed and at this level no antibiotic has been found in the blood serum or body tissues of chickens. At disease prevention or treatment levels of 50 to 200 gm./ton the antibiotic can be found in serum and body tissues but persists for only about 1 day after withdrawal.

The problem of a residue in poultry and swine is becoming limited where there is an increased tendency to use bacitracin or streptomycin that are not absorbed from the G.I. tract, or penicillin that is rapidly and quantitatively excreted.

Chlortetracycline and tetracycline appear in the bone and bone marrow after oral or parenteral administration. The antibiotic is apparently unaltered and permanently bound there in metallic combination in the newly formed bone. These antibiotics in high concentration impart a yellow color to the newly formed bone and smaller quantities can be detected by their ultra-violet fluorescence. It can only be eluted from the bone tissue by means of weak acid or EDTA. Cycline antibiotics are also bound by peptides in certain tumor tissues (23,24,25).

Metabolism

Penicillin: Penicillin was found to be bound to serum proteins and was isolated electrophoretically with them. The same amount of penicillin per unit of nitrogen was found in all protein fractions but fractions containing no nitrogen contained no penicillin. Penicillin added to human or guinea pig serum in vitro combines quantitatively with the serum proteins (26,27).

Penicillin labeled with radioactive S^{35} was given intramuscularly to cats and 100 percent of the radioactivity was recovered in the urine but simultaneously only 50 percent of the biological activity was so accounted for. When given orally 38 percent of the radioactivity was found in the urine within 8 hours while only 20 percent of the biological activity was found in the urine. The majority of the radioactivity remained in the G.I. tract (28).

Dibenzylethylenediamine (DBED) penicillin G labeled with C^{14} in the DBED moiety or the phenylacetyl moiety was found to remain in the body and at the site of the injection for longer than 3 days in rats, while penicillin G remained there for only 8 to 12 hours. In one trial more radioactivity from the intramuscular administration was found in the intestine than in the urine. Following oral administration 18 to 22 percent of each of the penicillins was excreted in the urine within 24 hours.

No labeled carbon was found in the respiratory CO_2. Total urinary excretion of biologically active material ranged from 30 to 60 percent of the I.M. dose. Only 2 percent of the phenylacetyl groups were split from the penicillin molecule and the major degradation product was penicilloic acid, a compound obtained in vitro by alkaline cleavage of penicillin (29).

This evidence indicates that penicillin or its metabolites are not stored in the body but are excreted, the rate depending on the reactivity or solubility of the antibiotic compound.

Cyclines: Oral administration of 250 mg. of C^{14} labeled oxytetracycline to humans produced little correlation between results of radiochemical and microbiological analysis on the same samples of arterial or venous blood, urine, or bile. Radioactivity was found in the blood serum and the urine for 7 days but biological activity was found in the serum for only 8 hours and in the urine for only 4 days. Evidently both urine and blood serum contain cycline metabolites that exert little or no microbiological activity (30).

Other Antibiotics: The liver in dogs has been shown to form a metabolic product from erythromycin that is still active microbiologically which is excreted in the bile but not reabsorbed as is the parent compound.

Carbomycin is apparently almost completely metabolized or inactivated since only 1 to 2 percent of the dose given by any route of administration can be accounted for (5).

It has been shown that a biologically inactive compound, isochlortetracycline, is formed as a result of the cooking of meat containing chlortetracycline. This compound has also been isolated from the G.I. tract after oral administration of chlortetracycline (22).

Present data give some information on the fate and metabolism of penicillin and cycline antibiotics but even this is meager and inadequate. The fate and metabolism of other antibiotics are as yet unexplored.

Excretion

The principal pathways of excretion are the urine and feces which have been discussed in all previous sections of the manuscript. The extent of urinary excretion was found to vary among individuals but the various antibiotics have general trends in this regard. After parenteral administration the recovery rate in the urine averages about 70 to 80 percent for penicillin, 60 percent for chlortetracycline, about the same for other cyclines, 10 to 30 percent for large doses and up to 100 percent for small doses for bacitracin, 50 to 90 percent for streptomycin in humans but 100 percent in dogs, 90 percent for chloramphenicol, 30 to 50 percent for neomycin, and 0.5 to 2 percent for erythromycin and carbomycin. After oral administration more oxytetracycline can be found in the urine than for chlor- or tetracycline (22 to 40 percent vs. 10 to 20 percent). Urinary recovery rates for some of the other antibiotics are: Penicillin 13 to 34 percent, 5 to 30 percent for chloramphenicol, and zero or very little for bacitracin, streptomycin, neomycin, and carbomycin. Except for the cyclines bound to bone and the metabolic fate of the portions not accounted for are unknown.

After intravenous administration, a serum half-life of over 2 hours was found for novobiocin, chlor- and tetracycline; 1 to 2 hours for streptomycin, erythromycin, and oleandomycin; and 0.5 to 1.0 hour for oxytetracycline and penicillin V or G (32).

Stability

Chlortetracycline is unstable in body fluids and as much as 88 percent may be inactivated in 1 hour when exposed to serum in vitro (5,7). The fact that urinary excretion may occur for 70 hours after the last dose indicates that in vivo deterioration must be somewhat less rapid than would be expected from the in vitro results. Oxytetracycline is stable in the presence of various body fluids in vitro. The stability of others has been discussed in the text.

The antibiotics that are used most frequently in animals have been shown to be stable or not inactivated by their passage through the G.I. tract; however, chloramphenicol is destroyed or inactivated in the tract. Their stability in feces after voiding is not known.

Effect of cooking meat containing cycline antibiotics: The average amount of cooking destroys the chlortetracycline that is found in chicken meat resulting from feeding high levels of antibiotic. Cooking also destroys all or practically all the cycline antibiotic residue in meat after post slaughter treatment which has been used as an aid in preservation.

REFERENCES

1. E. Jawotz. Polymixin, Neomycin, Bacitracin. 96 pp. Medical Encyclopedia Inc., N. Y. 1956.
2. M. H. Lepper. Aureomycin (chlortetracycline). 156 pp. Medical Encyclopedia Inc., N. Y. 1956.
3. M. M. Musselman. Terramycin (oxytetrocyline). Medical Encyclopedia Inc., N. Y. 1956.
4. S. A. Waksman. Neomycin. Rutgers Univ. Press. 1953.
5. H. Welch, et al. Principles and practice of antibiotic therapy. 699 pp. Medical Encyclopedia Inc. 1954.
6. J. M. Pensack, C. N. Huhtanen, and E. L. R. Stokstad. Laboratory evaluation of methods for potentiating antibiotics. Am. Cyanamid Co. 10th Animal Feed Symposium. pp. 33-64. 1959.
7. R. G. Brown and H. S. Bachman. Physiology and pharmacology of the potentiation of aureomycin. Ibid. pp. 93-104.
8. J. S. Kiser. Increasing the effectiveness of antibiotics as chemotherapeutic agents. Ibid. pp. 5-14. 1959.
9. R. E. Eggert and R. F. Elliot. The potentiation of Aureomycin in swine. Ibid. pp. 65-92. 1959. J. An. Sci. 18: 1505-1959.
10. K. E. Price, Z. Zolli, J. C. Atkinson, A. P. Collins, and H. G. Luther. Antibiotic inhibitors III. Reversal of calcium inhibition of intestinal absorption of oxytetracycline in chickens by certain acids and acid salts. Antibiotics Annual 1958-59: 1020-32.
11. G. Van Helsema, et al. The importance of using more than one animal species for blood level determination. Antibiotics Ann. 1955-59: 418.
12. L. L. Rusoff, H. H. Hester, Jr., and F. L. Landagore. Concentration of chlortetracycline in the body of dairy calves receiving nutritional levels of the antibiotic. Antibiotic Ann. 1954-55: 348. J. Dairy Science 37:488. 1954.
13. P. B. English. Serum penicillin concentrations in the bovine with fortified benzathine. Austral. Vet. J. 35:353. 1959.

14. J. K. Loosli and R. G. Warner. Antibiotics for dairy animals. Farm Research N. Y. State Agr. Exp. Sta., Quart. Bull. 18. 1952.

15. L. L. Rusoff and M. O. Haq. Studies on Aureomycin and vitamin B_{12} supplementations for dairy cows. II. J. Dairy Sci. 37: 677. 1954.

16. W. H. Martin, T. J. Clayton, and E. E. Bartley. Aureomycin content, bacterial development, starter activity and cheese quality of milk from cows fed an Aureomycin supplement. J. Dairy Sci. 38: 47. 1955.

17. A. L. Shor, W. P. Johnson, and A. Abbey. Effects of various amounts of chlortetracycline in the rations of lactating dairy cattle. J. Dairy Sci. 42: 1203. 1959.

18. C. G. Durbin, J. J. DiLorenzo, W. A. Randall, and J. Wilner. Antibiotic concentration and duration in animal tissues and fluids. II. Chicken blood, tissue and eggs. Antibiotics Ann. 1953-54: 428.

19. J. M. Pensack, A. A. Baldwin, R. C. Klussendorf, G. H. Crag, and D. C. Redd. Antibiotic feeding: relationship to tissue retention and bacterial resistance. Antibiotics Ann. 1953-54: 404.

20. H. G. Luther, W. M. Reynolds, J. R. McMahan, and R. C. Kersey. Antibiotic carryover in tissues of livestock. Antibiotics Ann. 1953-54: 416.

21. H. P. Broquist and A. R. Kohler. Studies of the antibiotic potency in the meat of animals fed chlortetracycline. Antibiotics Ann. 1953-54: 409.

22. R. J. Shirk, A. R. Whitehill, and L. R. Hines. A degredation product in cooked chlortetracycline-treated poultry. Antibiotics Ann. 1956-57: 843.

23. R. A. Milch, et al. Bone localization of tetracycline. J. Natl. Cancer Inst. 19: 87. 1957.

24. Boiko, V. I. Localization of chlortetracycline in bone tissue as determined by fluorescence. Antibiotics 4: 296. 1959.

25. T. L. Loo, E. D. Titus, and D. P. Rall. Nature of fluorophore localizing in tetracycline-treated mouse tumor. Science 126: 253. 1957.

26. K. Oeff, S. Rust, E. Schwarz, and H. J. Weise. The binding of penicillin to serum proteins. Klin. Wochschr. 33: 419. 1955. Naturwissenschaffen, 41: 500. 1954.

27. I. L. Marner and E. Lund. Examination of penicillin concentration in electrophoretically separated groups of serum proteins. Acta Path. Microbiol. Scand., 40: 267. 1957.

28. S. Rowlands, D. Rowley, and H. C. Stewart. Absorption and excretion studies with radioactive penicillin. Lancet, 1948 II: 493.

29. S. S. Walkenstein and J. Seitter. Fate and sojourn of C^{14} - Dibenzylethelenediamine (DBED) and C^{14} - DBED dipenicillin G following oral and intramuscular injection. Fate and sojourn of dibenzylethylenediamine C^{14} dipenicillin G and C^{14} penicillin G and C^{14} penicillin G following oral and intramuscular injection. Antibiotics Ann. 1953-54: 108; Antibiotics and Chemo. 4: 700. 1954. Ibid. 4: 1245. 1954.

30. C. M. Leevy, M. R. Zinke, and W. Y. Chey. Observations on the distribution of C^{14} oxytetracycline in man. Antibiotics Ann., 1958-59: 258.

31. C. C. Lee, R. C. Anderson, H. L. Bird, Jr., and K. K. Chen. Reabsorption of erythromycin and a microbiologically active metabolite in the bile of dogs and rats. Antibiotics Ann. 1953-54: 493, 485.

32. K. H. Spitzy and G. Hitzenberger. The distribution volume of some antibiotics. Antibiotics Ann. 1957-58: 996.

Absorption

The rate of absorption of diethylstilbestrol (stilbestrol) from pellets implanted in cattle has not been extensively studied. Hammond and Day (16) reported absorption data from diethylstilbestrol and Hexestrol tablets which were calculated from the difference in average weight of implanted and recovered tablets. Rates of absorption from tablets of 15 mg. to 1,000 mg. were rather constant and centered about 0.30 percent per day. Cowie and Flux (6) reported an absorption of 0.38 mg. per day (0.38 percent) for a 100 mg. tablet of stilbestrol implanted subcutaneously. When 15 of these pellets were implanted the amount absorbed increased to 4.97 mg. per day, but the rate of absorption was not considerably different, being 0.33 percent per day. More recently, Hale, et al. (15) studies the rate of absorption of 12 mg. stilbestrol pellets in ears of steers. Pellet residues were removed 28, 56, 84, and 112 days after implantation and the rate of absorption was found to follow the rate of a first order reaction. The half-life of the best pellet of the three studied was 73 days. The amount of stilbestrol absorbed daily was 0.073 mg.

The rate or absorption of stilbestrol pellets in poultry has received more attention. Absorption rate is greatest early in the treatment period and falls off with time, perhaps due to a decrease in surface area or encapsulation of the pellet. In one experiment Lorenz (19) reported an absorption of 3 mg./week from a 25 mg. stilbestrol pellet implanted subcutaneously or a rate of 1.64 percent per day. A later report (20) showed an average absorption of 2 mg./week from 20 to 25 mg. pellets, or a rate of 1.5 percent per day. This absorption rate fell to 0.75 percent per day after 4 weeks (21). Jones and Deatherage (17), using 15 mg. pellets, found an average of 3.97 mg. of unabsorbed stilbestrol remaining after 4 weeks of implantation. This absorption rate calculates as 2.6 percent per day. Stob, et al. (31) found an absorption of about 3 mg. per week from 12 mg. stilbestrol pellets during the first 2 weeks (absorption rate of 3.7 percent per day) of treatment. After 4 weeks only about half of the 12 mg. pellets were absorbed, and calculations for absorption would indicate 1.5 mg. per week or 1.8 percent per day instead of the higher early rate.

Vondell (41) also reported that about 1/2 to 1/3 of the original pellet was present after 6 weeks of stilbestrol treatment. The pellets were imbedded in tissue at this time. Recently, Umberger, et al. (39) recovered unabsorbed pellets after 5 weeks implantation of a 12 mg. pellet. The average residue weighed 6.3 mg. indicating an average absorption rate for 5 weeks of 1.4 percent per day. A comparison of the absorption rates of stilbestrol pellets in cattle (ca 0.40 percent per day) with those in poultry (ca 1.5 to 2.0 percent per day) indicates a rate four to five times greater in poultry.

The obvious hazard in the use of implants is that (detectable amounts of) residual estrogens may be present in the tissues of the treated animal. Studies on stilbestrol residues in meats and tissues, particularly with the use of oral administration of them, has shown that stilbestrol must be discontinued at least 48 hours before slaughter to provide a safe interval for clearance from the body. This is clearly not practical with stilbestrol implants.

In a recent study using tritium labeled hexestrol injected subcutaneously into lambs and goats, Glascock (11) found a very rapid absorption from the site of injection. Only a few percent of the original activity could be determined after a few hours.

The rate of absorption of orally administered stilbestrol, which is by far the most common route of administration, is difficult to assess quantitatively. Rapid excretion into the feces and urine has been noted by Mitchell, Neumann, and Draper (26), who found the appearance of radioactivity in the urine in the 0 to 6 hour interval, and in the feces in the 6 to 12 hour interval after the oral administration of tritium labeled stilbestrol. This

rather indirect evidence tends to indicate that absorption, metabolism, and excretion of orally administered stilbestrol probably is as rapid as the absorption of any ingested lipoid material.

Information on the absorption of stilbestrol or other synthetic estrogens when administered to poultry by routes other than implantation are very fragmentary. Experiments involving the use of pastes, stilbestrol-coated sugar pellets, solutions of stilbestrol in oil, or oral administration of estrogens, have yielded no direct quantitative information concerning the rate or amount of absorption of the active compound. Indirect evidence of absorption, or lack of it, is usually obtained from physiological effects. It has been concluded by this type of reasoning that the estrogens are absorbed too quickly and that the estrogenic effects have been too transitory to produce useful commercial responses (21).

Metabolism

Virtually nothing is known about the metabolism of diethylstilbestrol by the tissues of the body. While the enzymatic metabolism of the natural estrogens has been studied extensively and a metabolic scheme proposed and verified in many aspects, the corresponding information concerning stilbestrol is not known.

Pathways of excretion

Some information is available concerning the pathways of excretion of diethylstilbestrol. In nonruminants, the excretory products of stilbestrol have been determined. Three groups of workers have isolated and identified the conjugate of stilbestrol in rabbit urine as stilbestrol-mono-glucuronide (22, 10, 43). Hexestrol and dienestrol mono-glucuronides were also isolated. No evidence for the formation of an ethereal sulfate or mercapturic acid derivative was found. It therefore appears as if the main metabolic change of the three synthetic estrogens is conjugation with glucuronic acid without any change in the structure of the original compound. From 20 to 70 percent of the administered estrogen can be isolated in this conjugated form in the urine. Mitchell (26) using tritium labeled stilbestrol found that excretion in urine began within the first 6 hours after feeding. Terminal excretion times for radioactivity in the bile and the urine indicated that the period required for elimination of stilbestrol from the tissues of steers is about 72 hours. Radioactivity was detectable in the feces until 132 hours. When 10 mg. stilbestrol per day was fed to a steer for 11 days, 28 percent was recovered in the feces and 18 percent was recovered in the urine. Seventy-three percent of the activity in the urine, and 90 percent of the activity in the bile was found to be present in a conjugated form. In the feces, however, 98 percent of the activity was present as the free compound, which might indicate hydrolysis by microbial action of the conjugate. The activity in the kidney and liver was also present in the free form, 67 and 100 percent, respectively.

There have been a number of other reports on the relative amounts of stilbestrol recovered in the feces and/or urine after oral feeding. Rueber (29) found 44 percent of stilbestrol intake eliminated in the feces. Melampy et al. (23) recovered an average of 63 percent in the urine and feces. Story, et al. (33) recovered 80 percent of orally administered stilbestrol in lambs, 48 percent being found in the feces while 32 percent was recovered in the urine.

The recent work of Glascock (11) using tritium labeled hexestrol in lambs and goats gives the most complete and most accurate information on excretion reported to date. In these experiments almost all of the administered activity was recovered. The bile was the major pathway of excretion. The next major route was the urine. The maximum blood level of circulating estrogen was reached 2 hours after injection when about 22 percent of the dose was present in the circulation. Complete clearance from the blood was achieved within 24 hours. The injected hexestrol rapidly passed into the excretory pathways. The intestinal contents plus bile and feces contained approximately 37 to 45 percent of the activity, and the urine 25 to 30 percent, 5 to 8 hours after injection.

It appears from data on rat and rabbit, as well as some ruminant experiments, that the synthetic estrogens probably undergo an enterohepatic circulation. The liver conjugates the diethylstilbestrol as the monoglucuronide and secretes it into the bile. From the bile it is excreted into the intestine and a reabsorption from the intestine and recirculation appears likely. The feces represents the major pathway of excretion but the urine also accounts for a considerable part of the excreted material.

Very little is known about the pathways of excretion of the synthetic estrogens in poultry. Bird, Pugsley, and Klotz (3) found that 97.5 percent of the ingested dimethyl ether of stilbestrol could be recovered in the feces but only about 5.6 percent of dienestrol could be accounted for. When stilbestrol was fed, recovery in the feces was also very low. The recovery of the dimethyl ether of dienestrol was high, indicating a marked difference in metabolic behavior of the ethers as compared to the free phenols. The authors concluded that most of the dimethyl ethers of the estrogens are excreted directly and that the material absorbed from the pellet has the property of being absorbed into the lymph system thereby escaping passage through the portal field with consequent destruction in the liver.

Accumulation in Tissues and Body Fluids

The accumulation of hormone residues in the tissues and body fluids has received a great deal of attention because of the possible hazard to humans ingesting the meat of these animals. A number of factors have complicated the problem of determining the tissue retention of estrogenic residues. Route of administration, time between hormone treatment and killing of the animal, and the method of detection are all factors which have been responsible for certain conflicting results in the literature (7). The advent of more sensitive methods of assay and detection and better designed and controlled experiments have now resulted in more uniform agreement between investigators.

Implantation studies have yielded conflicting tissue residue data. Thus, Stob, et al. (31) found small amounts of estrogenic activity by bioassay in beef and lamb muscle and liver which did not exceed 0.01 μg per gram of dried tissue, when 12 mg. or 24 mg. implants were used. Swift (34) by using a chemical method of detection did not detect any stilbestrol residues in muscle, liver, or fat of steers or lambs. Ellis, et al. (8) detected estrogenic activity in muscle, liver, and kidney fat of lambs implanted with stilbestrol. Anderws, et al. (1) detected small residual estrogenic activity in tissues from lambs injected subcutaneously with stilbestrol.

Investigations involving the use of orally fed stilbestrol have shown that the time interval between the removal of stilbestrol-feed and slaughter is of paramount importance in ensuring residue-free tissues. Wiberg and Stephenson (42) found no biological activity when the stilbestrol-feed was discontinued 48 hours before slaughter, but detectable levels were found in lean meat, liver, and kidney when the time interval was reduced to 24 hours. A careful study on the residual estrogenic activity of tissues by Umberger, et al. (38) reaffirmed the fact that no detectable residues were present in tissues of steers which were slaughtered 48 hours after the last feeding of DES. This finding was in complete agreement with similar studies by Perry (27), Turner (37), Preston (28), and Gossett (12). A report by Beeson, et al. (2) showed no estrogenic activity in the meat from stilbestrol fed pigs. Davey, et al. (9) studied the use of oral stilbestrol in lamb feeding and found estrogenic activity in one trial but no activity in another trial. Stob, et al. (32) found tissue levels when the animals were fed stilbestrol right up to the time of shipment for slaughter.

When tritium labeled stilbestrol was fed, Mitchell (26), thereby permitting the detection of very small quantities of material, very low levels were found in lean meat (0.30 p.p.b.), internal fat (0.35 p.p.b.), and somewhat higher quantities in liver (9.12 p.p.b.) and kidney (4.15 p.p.b.). These animals, however, were slaughtered only 27 hours after discontinuing the stilbestrol feed. The data indicated, however, that a period of about 72 hours would be required for the complete elimination of stilbestrol from the tissues.

Glascock (11), using tritiated hexestrol in lambs and goats, also reported measurable amounts of radioactivity in tissues after 24 hours and even after 48 hours. Many tissues were examined and accumulation was found in almost all tissues and organs. About 1.5 percent of the administered activity remained in the body tissues after 24 hours and only about 0.8 percent after 48 hours. The use of this very high specific activity achieved by tritium labeling has resulted in the first confirmation of selective localization in reproductive organs, since the uterine endometrium, vagina, myometrium, oviducts, ovaries, and mammary glands were found to have higher relative concentrations than other tissues and organs.

There have been numerous studies on the presence of hormone residues in poultry tissues. In 1947 Bird, et al. (3) found that when the dimethyl ethers of stilbestrol (dianisylhexene), hexestrol (dianisylhexane), and dienestrol (dianisylhexadiene) were fed for 7 to 12 days the abdominal fat contained large quantities of estrogenic activity, 2.5 to 4.3 mg., 5 mg. and 1.5 mg., respectively, per 100 gm. of adipose tissue. Estrogenic activity was found in all the extracts of tissues of the cockerels fed the ethers of the estrogens or injected subcutaneously with these compounds. The muscular tissues were low in activity but skin, liver, gizzard, and abdominal fat showed high potency. When liver or fat from treated birds was fed to postmenopausal women, estrous changes were observed in their vaginal smears, indicating the high estrogenic content of the tissues. The above compounds have not been used commercially.

Tiger, Mitchell, and Goff (36) in 1956 found no detectable estrogen in extracts of skin, muscle, abdominal fat, and liver from chickens fed dienestrol diacetate (32 mg./lb. of feed). In 1959 Umberger and Gass (40) reported results very similar to those obtained by Tiger, et al. (36). When dienestrol diacetate was fed at the rate of 32 mg./lb. feed for 3 weeks and the estrogen feed replaced with a control mash 24 hours before killing, no estrogenic residues could be detected in muscle, abdominal fat, skin fat, heart, gizzard, or spleen. Estrogenic activity was found in liver (3 p.p.b.), kidney (12 p.p.b.) and small intestine (10 p.p.b.). The results suggested that dienestrol diacetate is not stored in the edible tissues, but a period of 24 hours off the estrogen feed is not sufficiently long to insure complete removal of the estrogen from the usual pathways of excretion.

Gowe (13) reported estrogenic activity of not more than 14.5 μg in the extracts of 100 gm. of muscle and skin and 5 μg in the extracts of 100 gm. of liver. No activity was found in turkey gizzards, hearts, and abdominal fat. In 1950 Colombel (5) reported large quantities of estrogen, 6,000 p.p.b. DES equivalents in chicken livers from birds castrated chemically with 15 mg. and 30 mg. pellets of stilbestrol. Meyer (25) determined 13 p.p.b. estrone equivalents in extracts of chicken carcass and 24 p.p.b. estrone equivalents in extracts of chicken livers after 12 mg. stilbestrol pellet treatment.

Campbell and Wick (4) in 1952 found no estrogenic activity in extracts of chicken carcass but determined 60 to 118 p.p.b. estrone equivalents in chicken livers after implantation of 12 mg. stilbestrol pellets. Turkey liver extracts from 12 mg. pellet implants also showed 50 p.p.b. estrone equivalents. Greenblatt and Brown (14), however, found no estrogenic activity in extracts of chicken fat from chickens treated with 15 mg. and 30 mg. pellet implants. Koch and Heim (18) found estrogenic activity in extracts from all tissues of chickens treated with 20 mg. stilbestrol pellet implants. Muscle, bones, skin, liver, and abdominal fat contained from 0.01 to 34 mg. per 2200 gm. bird.

Jones and Deatherage (17), using a chemical method for determining stilbestrol, failed to detect estrogen in extracts of liver, breast muscle, or abdominal fat. The colorimetric method was not sensitive to small quantities of estrogen however, and the authors acknowledged the limits of sensitivity in the conclusions in their report. Swift in 1954 also used a chemical method for estimation of stilbestrol residues in poultry tissues. There was no evidence of estrogen in extracts of breast muscle, leg or back muscle but extracts of liver and skin from cockerels treated with a 15 mg. pellet of stilbestrol contained 37 μg./gm. or approximately 0.25 percent of the amount implanted.

In 1954 Snair, et al. (30) stated that residues containing significant amounts of stilbestrol had been recovered from the fatty tissue of poultry after the pellet implantation of stilbestrol. Stob, et al. (31) reported measurable estrogenic activity which did not exceed 0.1 μg./gm. dried tissue after treatment of chickens with 12 mg. stilbestrol pellets for 1, 2, 3, or 4 weeks. They point out that this is a rather high level of hormone in comparison with sheep and cattle when considered on a dose per unit of body weight. In this assay for estrogens the dried tissues were directly incorporated into the diet of the test mice instead of preparing extracts of the hormone containing tissues.

Merker, et al. (24) did not detect any estrogenic activity in either the free or conjugated residues of extracts obtained from chickens which had been treated with 12 mg. stilbestrol pellets.

Umberger, et al. (39) also studied the estrogenic residues in the edible tissues of stilbestrol implanted chickens using a sensitive bioassay in which immature mice were fed the chicken tissues directly. Stilbestrol was applied to the chickens in either 12 mg. pellets, a 15 mg. paste, a 15 mg. liquid rosin, or 15 mg. liquid preparations. Measurable estrogenic residues were detected after the use of either of the first three methods of administration. No activity was detected 5 weeks after the use of the liquid preparation. The amounts of estrogenic activity were equivalent to 20 to 30 p.p.b. of stilbestrol in the liver and to 35 or more p.p.b. in the fat of the skin. Detectable amounts also occur in the kidneys and small intestines of the treated birds. Smaller amounts, 0.5 to 1.8 p.p.b., were found in the breast and leg muscles. In a review of the literature on estrogenic residues in poultry Umberger, et al. (39) pointed out that in only a few cases were the treated tissues fed to the assay animals; the other assays involved extraction of the estrogen from the tissues with attendant possibilities of losses.

The residual estrogens found in meat appear to be relatively stable to heat. Thus, Stob, et al. (31) found no losses after cooking. Taylor and Gordon (35) also found no destruction of estrogenic activity in swine tissues by cooking. Umberger, et al. (39) also found no losses in diethylstilbestrol content in chicken tissues due to cooking.

Stability of hexestrol in excreta appears to be high. A recent report (44) has shown estrogenic activity in silage contaminated with manure from estrogenized steers which persisted for several months.

REFERENCES

1. Andrews, F. N., Stob, M., Perry, T. W., and Beeson, W. M. J. An. Sci. 15: 575 (1956).
2. Beeson, W. M., Andrews, F. N., Perry, T. W. and Stob, M. J. An. Sci. 14: 475 (1955).
3. Bird, S., Pugsley, L. I., and Klotz, M. O. Endocrinology 41: 282 (1947).
4. Campbell, E. D., and Wick, H. Endocrine Laboratories Report pp. 1438-1439. (See Meyer, R. K. for ref.)
5. Colombel, J. Rec. de Med. Veterinaire Paris 126: 100 (1950).
6. Cowie, A. T., and Flux, D. S. J. Endocrinol. 11: 255 (1954).
7. Ellis, N. R., Davis, R. E., and Denton, C. A. Antibiotics, Hormones, Tranquilizers in Animal Production Symposium USDA April 27-29, 1960.
8. Ellis, P. J., Allen, C. E., and Whiting, F. Canad. J. Agr. Sci. 34: 292 (1954).
9. Davey, R. J., Armstrong, D. T., and Hansel, W. J. An. Sci. 18: 75 (1959).
10. Dodgson, K. S., Garton, C. A., Stubbs, A. L., and Williams, R. T. Biochem. J. 42: 357 (1948).
11. Glascock, R. F., and Hoekstra, W. G. Biochem. J. 72: 673 (1959).
12. Gossett, F. O., Smith -- and Downing -- Symposium on Medicated Feeds 1956, Washington, D. C.

13. Gowe, R. S. Poultry Sci. 28: 666 (1949).

14. Greenblatt, R. B., and Brown, N. H. Am. J. Gynec Obstet. 61: 1361 (1952).

15. Hale, W. H., Sherman, W. C., White, E. A., Kuhn, G., Schrell, R. B., Reynolds, W. M., and Luther, H. G. J. An. Sci. 18: 120 (1959).

16. Hammond, J. and Day, F. T. J. Endocrinol. 4: 53 (1944).

17. Jones, O., and Deatherage, F. E. Food Res. 18: 30 (1953).

18. Koch, W., and Heim, G. Endokrinologie 29: 288 (1952).

19. Lorenz, F. W. Poultry Sci. 22: 190 (1943).

20. Lorenz, F. W. Poultry Sci. 24: 91 (1945a).

21. Lorenz, F. W. Poultry Sci. 24: 128 (1945b).

22. Mazur, A., and Shorr, E. J. Biol. Chem. 144: 283 (1942).

23. Melampy, R. M., Gurland, J., and Rakes, J. M. J. An. Sci. 18: 178 (1959).

24. Merker, P. C., Edwards, L. D., Andrews, F. N., and Christian, J. E. Poultry Sci. 34: 1118 (1955).

25. Meyer, R. K. Endocrine Laboratories Report No. 1EL-270 (1952) In U. S. Congress. 82nd Congress 2nd Session Hearings H. Res. 74 and H. Res. 447, Part 3, Appendix B, pp. 1436-1438.

26. Mitchell, G. E., Neumann, A. L., and Draper, H. H. J. Agr. Food Chem. 7: 509 (1959).

27. Perry, T. W., Beeson, W. M., Andrews, F. N., and Stob, M. J. An. Sci. 14: 329 (1955).

28. Preston, R., Cheng, E., Story, C. D., Homeyer, P. G., Pauls, J., and Burroughs, W. J. An. Sci. 15: 3 (1956).

29. Rueber, H. W. Am. J. Vet. Res. 19: 585 (1958).

30. Snair, D. W., Jaffray, S. E., Grice, H. C., and Pugsley, L. I. Canad. J. Biochem. Physiol. 32: 41 (1954).

31. Stob, M., Andrews, F. N., Zarrow, M. X., and Beeson, W. M. J. An. Sci. 13: 138 (1954).

32. Stob, M., Perry, T. W., Andrews, F. N., and Beeson, W. M. J. An. Sci. 15: 997 (1956).

33. Story, C. D., Cheng, E. W., Pauls, J., and Hale, W. H. J. An. Sci. 16: 307 (1957).

34. Swift, C. E. Food Res. 19: 402 (1954).

35. Taylor, J. H., and Gordon, W. S. Vet Rec. 67: 48, 58 (1955).

36. Tiger, H. L., Mitchell, H. I., and Goff, S. Symposium on Medicated Feeds. Washington, D. C., p. 183 Ed. Henry Welch and Felix Marti-Ibanez Medical Encyclopedia Inc. N. Y. 1956.

37. Turner, C. W. J. An. Sci. 15: 13 (1956).

38. Umberger, E. J., Curtis, J. M., and Gass, G. H. J. An. Sci. 18: 221 (1959).

39. Umberger, E. J., Gass, G. H., Davis, K. J., Curtis, J. M., and Durbin, C. G. Poultry Sci. 38: 118 (1959).

40. Umberger, E. J. and Gass, G. H. Poultry Sci. 38: 128 (1959).

41. Vondell, J. H. Poultry Sci. 31: 174 (1952).

42. Wiberg, G. S., and Stephenson, N. R. Canad. J. Biochem. and Physiol. 35: 1107 (1957).

43. Wilder-Smith, A. E., and Williams, P. C. Biochem. J. 42: 253 (1948).

44. Rankin, J. E. F. Vet. Rec. 71: 924, 1959.

THYROXINE

Absorption from the Gastrointestinal Tract

The bulk of the earlier work on absorption of thyroxine and thyroxine-containing products has been largely concerned with the relative rates of absorption of thyroxine in its several forms. Little quantitative data on actual absorption were obtained. On the basis of comparisons of urinary iodine excretion with total thyroidal iodine administered, an estimated 16-28 percent absorption was suggested. Absorption from isolated intestinal loops of 90 percent and from isolated stomach of 40 percent have been observed. Recently Monroe and Turner (4) reinvestigated this problem by comparing the amount of orally administered thyroxine needed to maintain normal thyroid size in thiouracil-treated chicks with the amount required by parenteral administration. These tests indicated an absorption of 15 to 20 percent. These same workers using goats and comparing input with fecal output calculated absorption of 78-88 percent. When re-excretion of absorbed thyroxine into the gut is considered, this high value would suggest that thyroxine is almost completely absorbed from the gut in this species. Mixner and Lennon (3) have also estimated the efficiency of absorption of thyroxine from thyroprotein to be 8.3 to 17.4 percent.

Accumulation in Tissues

After parenteral administration, thyroxine appears to be distributed about equally between blood, liver, and the remaining body tissues. No quantitative data exist on actual concentration of thyroxine in tissues as affected by intake. The half-life of thyroxine in humans is estimated at 7 to 12 days. Thyroxine rapidly disappears from blood and the major points of accumulation are then in the liver, kidney, and intestinal tract. Presumably muscle and other edible tissues are in equilibrium with the blood and would reflect changes in thyroxine content of blood produced by administration of thyroxine. The feeding of thyroprotein containing thyroxine increased blood protein-bound-iodine values from a normal level of 2.0 to 5.0 μg./100 ml. to levels exceeding 25.0 μg./100 ml. (2). Administration of thyroxine containing products (iodinated casein) to cows apparently does not cause detectable accumulation in the milk. There is evidence, however, that thyroxine may be transmitted from mother to child during the suckling period (7). As much as 10 percent of the activity of labeled thyroxine was observed in the ovocytes of laying hens (9).

Metabolism of Thyroxine

The major known metabolic change in thyroid hormone is one of deiodination. Most of the liberated iodine is excreted in the urine. Deiodination appears to occur primarily in the liver and kidney. The disposal and the nature of the noniodinated residues are practically unknown. Presumably they could be metabolized to tyrosine or to propionic acid. Two propionic acid derivatives, tetraiodopropionic acid and tri-iodopropionic acid are present in small quantities in bile and urine but not in feces. Phenolic glucuronides of thyroxine and tri-iodothyroxine are also present in bile but do not appear in blood, urine, or feces. This latter mechanism apparently represents a detoxification reaction and also involves an entero-hepatic circulation of the thyroidally active components.

Excretion of Thyroxine

In experiments with laboratory animals from 50 to 80 percent of an injected dose of thyroxine appears in the gastrointestinal tract within 2 hours. The major pathway of entrance is by way of the liver and bile. Part of the thyroxine at least is reabsorbed. About two thirds is ultimately excreted in the feces as thyroxine mostly and the remainder metabolized and excreted as iodide in the urine.

REFERENCES

1. Bruger, M., and Silberbush, F. F. J. Clin. Endocrin. 6: 565, 1946.
2. Long, J. F., Hibbs, J. W., and Gilmore, L. O. J. Dairy Sci. 36: 1049, 1953.
3. Mixner, J. P., and Lennon, H. D. J. Dairy Sci. 41: 728, 1958.
4. Monroe, R. A., and Turner, C. W. Univ. Missouri Res. Bul. 446, 1949.
5. Rawson, R. W. Fed. Proc. 13: 663, 1954.
6. Reineke, E. P., and Turner, C. W. J. Dairy Sci. 27: 793, 1944.
7. Robertson, J. D. J. Endocrin. 4: 300, 1945.
8. Roche, Jean, and Michel, Raymond Physiol. Rev. 35: 583, 1955.
9. Roche, Jean, Michel, R., and Volpert, E. Compt. Rend. Soc. Biol. 150: 2149, 1956.

TRANQUILIZERS IN ANIMALS

During the last few years, a massive volume of literature has materialized on the therapeutic use of the tranquilizing agents. More recently, the value of these agents in animal nutrition has been tested, and a respectable volume of literature has already appeared in this limited field alone. In addition to Rauwolfia vomitoria and reserpine, the drugs tested in animal nutrition studies include: Chlorpromazine, hydroxyzine, perphenazine, prochlorperazine, tetrahydrozoline, trifluoperozine, and others.

In constrast with the massive existing literature on therapeutic and nutritional uses of the tranquilizers almost nothing has been published on tissue accumulation and metabolic fate of the drugs. An example of the paucity of information on the subject is given by the National Research Council Handbook (on) Tranquilizers (1), published early in 1959. While information on 26 tranquilizing drugs is given by the handbook, metabolic information is given on only three, as follows: (1) "Chlorpromazine is known to be converted, in part, to the sulfoxide, but the major metabolites are still unknown." (2) "Iproniazid is rapidly split into isopropyl hydrazine (the active moiety) and isonicotinic acid, the latter being promptly excreted in the urine. The long duration of action of iproniazid as an amine oxidase inhibitor may be accounted for by the attachment of the isopropyl hydrazine moiety to the amine oxidase." (3) Meprobamate "A portion of the drug is found in urine as a conjugate with glucuronic acid, and 10% is recovered unreacted. The major metabolites of meprobamate are unknown." During the year that has elapsed since the publication of the handbook, relatively little additional information has been published on this subject.

Excretion

Such information as has been published on the metabolism of the tranquilizers seems to be, to some extent, mutually contradictory. An early contribution to knowledge of the rate of excretion of chlorpromozine from the body was made by Dubost and Pascal in 1953 (2). These workers reported that following oral doses ranging from 0.25 to 0.5 gm. per kg. of bodyweight, the blood level of the drug in man is 4 to 8 mg. per liter after 3 hours, dropping to 2.4 to 4 mg. per liter after 6 hours, and to 1.7 to 2.5 mg. per liter after 24 hours. Only 7 to 8 percent of the ingested drug appears in the urine. Given subcutaneously in doses of 0.1 to 0.25 gm. per kg., the blood concentration of chlorpromazine falls more rapidly than it does when administered by mouth. Reports vary as to the percentage of chlorpromazine excreted through the kidney. Berti, et al. (3) reported that in the rat, guinea pig, rabbit, and man 20 percent of the dose leaves the body in 4 days while the percentage eliminated in this way by the dog is twice that of the other species studied. The excretion rate appeared to be independent of level of dosage

or route of administration. Others (4) report that urinary excretion is 20 percent with a daily dose of 75 mg., but that this declines to 5 percent when the dosage level reaches more than 400 mg. daily. Total excretion, however, remains at 25 mg. per 24 hour period within a dosage range of 200 to 400 mg. daily. The percentage level of urinary excretion of levomepromazine is around 12 percent with daily doses of 17, 100, and 125 mg.

It has recently been reported (5) that in cattle, approximately 12 percent of intravenously injected doses of 0.19, 0.25, and 0.4 mg. of chlorpromazine hydrochloride per pound of bodyweight was excreted in the urine during the 24-hour period following injection.

It has been reported (6) that chlorpromazine can be found in the urine of humans for 4 to 12 days following withdrawal and this variation does not depend upon dosage level alone. It has been demonstrated (7) that following doses as massive as 1,000 mg. per day, the blood of humans does not contain more than 0.4 mg. per 100 cc., while not even a trace is present in the cerebrospinal fluid. Only 2 to 4 percent appears in the urine in unchanged form whether the material is injected or given by mouth.

Accumulation in Tissues

Concentration levels of chlorpromazine in the tissues following treatment vary widely. Kok (8) reports that following a subcutaneous injection of 150 μM[2] per kg. into rabbits, tissue concentrations in μM per liter of blood or per kg. of tissue were as follows: Blood, 3.6; liver, 30; lung, 470; skeletal muscle, 11; brain, 51; and heart, 45. In a recent investigation (9) chlorpromazine concentration was found to be 520 mg. per kg. of liver and 90 mg. per kg. of brain following intramuscular injection into rats of 25 mg. of drug per 100 gm. of bodyweight. In another investigation (10) it was found that chlorpromazine accumulates in unequal concentrations in the various parts of the brain.

In cattle slaughtered 8 hours after injection of 0.4 mg. chlorpromazine per lb. bodyweight, small residues were found in the fat, brain, heart, lung, and kidney of the animals. In cattle slaughtered 72 hours after intravenous injection, no chlorpromazine could be detected. No chlorpromazine was found in the muscle of treated animals (5).

Metabolism

Only about 1 percent of a 20 mg. per kg. bodyweight intravenous dose of chlorpromazine injected into dogs appears unchanged in the urine according to Salzman et al. (11), while 12 percent appears as the sulphoxide. When this metabolite is injected, only 20 percent appears unchanged in the urine. They also report (12) that two metabolites other than the sulphoxide are present as is shown by chromatography. The drug disappears relatively slowly from the tissues, the amount remaining 7 hours after injection being of the same order as that remaining after 1 hour. However, the small amount of the drug found in the plasma at 1 and 3 hours disappeared by the seventh hour.

An important means of chlorpromazine metabolism detoxification in man is hydroxylation followed by conjugation with glucuronic acid according to Lin, et al. (13). Young, et al. (14) reported the recovery of 40 percent of the radioisotope of C^{14} chlorpromazine added to rabbit and rat liver homogenates as $C^{14}O_2$ demonstrating that the liver is at least able to lower the pharmacological activity of the drug. The appearance of $C^{14}O_2$ in air expired by rats given labeled chlorpromazine has been demonstrated by Ross (15). This is evidence that the side chain is metabolized. From one group of rats given 15 mg. of the drug per kg. bodyweight by mouth, 17 percent of the isotope was recovered from expired air during the first 6 hours following administration. A second group of rats pretreated with daily doses equal to the labeled dose of the drug for 10 days prior to the oral administration of 15 mg. of radioactive chlorpromazine released 12 percent of the administered isotope in 6 hours. Rats given 2 mg. of the drug per kg. bodyweight intravenously also released 12 percent of the radioactive material in 6 hours.

[2] μM is equivalent to micronole, or 1 millionth of the molecular weight of a given compound.

REFERENCES

1. Grebe, Rudolph M. Handbook of Toxicology, Volume IV, Tranquilizers, WADC Tech. Rpt. 55-16, Volume IV, ASTIA Document No. AD 211697, 1959.

2. Dubost, Paul, and Pascal, Suzette. Annales Pharmaceut. Franc. 11, 615-620. 1953.

3. Berti, T., and Cima, L. Il farmacs. ed. sc. 12: 159-165, 1957.

4. Nadeau, Guy, and Sobolewski, Georges. Canad. Med. Assoc. J. 81: 658-9, 1959.

5. Hendrickson, R. L., Odell, G. V., et al. J. An. Sci. 19: 26-33. 1960.

6. Neve, Hans K. J. Ment. Sc. 104, 488-490. 1958.

7. Kimberger, Björn Arne. Psychopharmacology frontiers. Editor Kline, Nathan S. Ch. 59, Little, Brown and Company, 1959.

8. Kok, K. Acta Phys. Pharmacol. Neerlandica 4: 388-394, 1955.

9. Wechsler, Magdalenea Berger, and Forrest, Irene S. J. Neurochem. 4: 366-371, 1959.

10. Wase, Arthur W., Christensen, Jens, and Palley, Edward. Arch. Neural. and Psychiat. 75: 54-6, 1956.

11. Salzman, Norman P., Moran, Neil C., and Brodie, Bernard B. Nature 176: 1122-3, 1955.

12. Salzman, Norman P., and Brodie, Bernard B. J. Pharmacol. and Exp. Therap. 118: 46-54, 1956.

13. Lin, T. H., Reynolds, Luther W., et al. Proc. Soc. Exp. Biol. and Med. 102: 602-5, 1959.

14. Young, R. L., Ross, Jr., J. J., and Maass, A. R. Nature 183: 1396-7, 1959.

15. Ross, Jr., John J., Young, Richard L., and Maass, Alfred R. Science 128: 1279-80, 1958.

16. Voelker, H. H., and Fitzgerald, M. S. Dakota Farm and Home Research 11: 7-9.

☆ U. S. GOVERNMENT PRINTING OFFICE : 1960 O - 563458